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HEMOGRAPHY IN THE DIAGNOSIS OF APPENDICITIS BASED ON 500 CASES*

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IN RECENT years there has been an attempt to obtain more information from the leucocyte count and the stained blood smear than is afforded by the simple Ehrlich differential count. A number of methods for classifying the neutrophiles have been offered, among the best known being those of Schilling and of Arneth.

The Schilling method of dividing the neutrophiles into four groups based on their maturity, i.e., myelocytes, juveniles, stabs and segmenters is in common use, being recognized as a more complete and valuable diagnostic aid than the conventional Ehrlich method and more simple than the complicated Arneth system.

It is logical that the Schilling principle should be applied to the differentiation of various types of appendicitis and we have, therefore, employed a modification of the Schilling hemogram.

Kohl uses only a part of Schilling's classification of neutrophiles to distinguish four degrees of appendicitis which he does not describe. He bases his diagnosis on the total percentage of immature neutrophiles (myelocytes + juveniles + stabs) which Schilling calls "shift cells," e.g.,

SHIFT CELLS	APPENDICITIS
11-25%	First degree
25-45%	Second degree
45-60%	Third degree
60 and above %+	Fourth degree

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In appendicitis, as in all infections, it is not sufficient to consider the shift cells alone in making a diagnosis from the hemogram. This one item becomes more important when considered in its relation to the total leucocyte count, the percentage of segmenters, total neutrophiles, lymphocytes, monocytes, etc.

We believe that the entire hemogram, when carefully analyzed and considered with the total number of shift cells, affords the most accurate differentiation. It is possible, we believe, to recognize the various stages of appendicitis from the most quiescent, fibrotic state to the most active, diffuse inflammatory process.

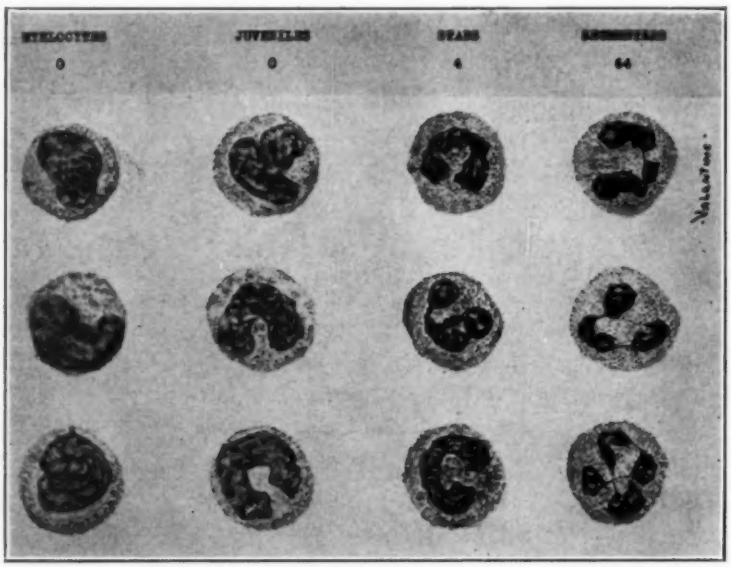

Fig. 1.—Staining with Wright's stain and buffer solution. (1) Wright's stain one minute. Cover the slide with stain, 10 to 15 drops. (Cells fixed by the methyl alcohol and staining begun.) (2) Buffer solution three minutes. Add an equal quantity of buffer solution to the wright's stain on the slide, 20 to 30 drops. (Staining completed.) (3) Wash in distilled water. (4) Blot. (5) Decolorize in 95 per cent alcohol one second to remove stain deposit. (6) Wash in distilled water immediately until the slide stops streaking. This must be done quickly to stop the decolorizing action of the alcohol. (7) Blot.

Wright's stain, Biosal Products Co., Rochester, N. Y., 1 tablet to 10 c.c. of absolute methyl alcohol C. P.

Buffer Solution pH 6.45.

Stock Sol. 1. 28 c.c.

Stock Sol. 11. 72 c.c.

Dist. H_2O 900 c.c.

Stock Sol. 1. $Na_2PHO_4.2H_2O$ (11.87 grams dissolved in 1,000 c.c. distilled water) $Na_2HPO_4.2H_2O$ is obtained by spreading $Na_2HPO_4.12H_2O$ in a thin layer and drying in the air for two weeks.

Stock Sol. 1. $Na_2HPO_4.2H_2O$ (11.87 grams dissolved in 1,000 c.c. distilled water)

HEMOGRAPHY

We have modified the Schilling hemogram by differentiating the lymphocytes on the basis of their maturity, in addition to the neutrophiles, and by adding several indices. For detailed information our previous paper* may be consulted.

*Crocker and Valentine: Hemography in Diagnosis, Prognosis and Treatment, Based on 6000 Schilling Hemograms, J. LAB. & CLIN. MED. 20: 172, 1934.

Normally there are in the peripheral blood approximately 5,000 neutrophiles per cubic millimeter distributed as follows according to Schilling:

myelocytes	juveniles	stabs	segmenters	total neutrophiles
0	0	4	64	68

Schilling calls myelocytes, juveniles and stabs, "shift cells." Except for 4 to 10 per cent stabs these cells are absent from the peripheral blood during health, but may appear in varying numbers during infections and intoxications. An increase in the number of shift cells with a decrease in segmenters is called a shift to the left, and indicates that the severity of the infection is increasing. A decrease in the number of shift cells, with an increase in segmenters, is a shift to the right and indicates subsidence of an acute or subacute infection or may possibly be due to a low grade chronic intoxication.

The quantity of the shift is expressed by the Schilling index and the multiple index. The Schilling index is the ratio of the total shift cells to segmenters, e.g.,

M		J		St		Sg	
0	+	0	+	4	:	64	4/64 or 1/16, normal Schilling index, no shift.
M		J		St		Sg	
6	+	18	+	48	:	24	72/24 or Schilling index of 3, wide left shift.

The "multiple index" is the Schilling index multiplied by 16 or the Schilling index expressed somewhat differently. The normal multiple index is therefore $\frac{1}{16}$ times 16 or 1. While these indices have the same relative value they are expressed differently for convenience in analyzing the hemogram. The Schilling index gives the ratio of immature to mature neutrophiles. The multiple index shows the number of times the shift is greater than normal and therefore may indicate the degrees of infection or intoxication.

The quality of the left shift is determined by the type of shift cells appearing in the peripheral blood. Myelocytes, juveniles, and stabs are found in varying numbers depending on the severity and type of the infection or toxic agent. These cells are found in the bone marrow normally but are probably not sufficiently mature to pass the threshold into the peripheral blood. Increasing toxic depression of this threshold permits the escape of the immature neutrophiles from the bone marrow in the order of their maturity as though they were formed in layers. The reserve segmenters pass the threshold first and are soon depleted, then the stabs, next the juveniles, and finally the myelocytes, thus extending the qualitative shift progressively to the left.

The qualitative shift must be considered with the quantitative shift as indicated by the Schilling index, since the latter gives no indication of the individual types of shift cells which are being allowed to pass the threshold. For a quantitative shift, or Schilling index of 3, the qualitative shift might be:

M	J	St	Sg			
0	0	60	20	=	60/20	or SHI 3
		or				
M	J	St	Sg			
6	24	30	20	=	60/20	or SHI 3

The quantitative shift is the same in both hemograms, but the quality of the shift is different in each. The first is a simple degenerative or stab left shift.

The latter is a leucemoid or myelocytic left shift with myelocytes and juveniles present in addition to stabs. It indicates a much greater depression of the threshold and a more severe intoxication. This information cannot be obtained from the quantitative shift or Schilling index alone.

The lethal index is qualitative in character. It is arbitrarily determined by obtaining the ratio of all the myelocytes, one-half the juveniles or one-eighth of the stabs to the segmenters. When it reaches one or over and the multiple index is 100 or above, the patient is certainly extremely ill, so ill that a fatal outcome is common.

By the term "threshold" we mean that mechanism which qualitatively controls the maturation and release of the cells which pass from the bone marrow into the peripheral blood during infections or intoxications.

The term "regenerative power" refers to the power of the bone marrow to increase the production of neutrophiles under toxic stimulation. In some cases "toxic pressure" may be so great that this ability is decreased or lost and production of neutrophiles is less than normal.

The hemogram is a horizontal tabulation of the various important items in the blood picture.

The shiftograph is a vertical graph of the Schilling indices of a chart and indicates the extent of the quantitative shift to the left or right, in relation to the qualitative shift. The direction and extent of the graph reveals the course of illness of the patient which can be roughly estimated at a glance.

The shiftogram we refer to as a chart of serial hemograms connected by a shiftograph, showing the entire course of the disease. Analysis from day to day may show extension or subsidence, convalescence or complication and frequently signs of approaching death. It may be a guide to medical and surgical treatment and probably constitutes a valuable indicator in determining improvement or retrogression of a patient under many forms of therapy in routine or research problems.

DESCRIPTION AND DIFFERENTIATION

Five hundred cases of appendicitis were studied clinically and by hemogram, and the following degrees could be differentiated:

First Degree or Chronic Fibrous Appendicitis.—With vague clinical symptoms and a history of recurrent attacks the hemogram of chronic fibrous appendicitis presents quite constant characteristics.

The white cell count usually remains within the normal limits of 5,000 to 10,000 per c.mm. The percentage of neutrophiles ranges from 40 to 70, total shift cells 10 to 35, lymphocytes 20 to 40, monocytes 1 to 15, eosinophiles 0 to 6, basophiles 0 to 5, lethal index $\frac{1}{40}$ to $\frac{1}{7}$, multiple index 4 to 16, Schilling index $\frac{1}{4}$ to 1, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 5 A.

The neutrophilic left shift is confined almost exclusively to stabs. It is always slight and with the neutropenia may point to an insidious absorption of toxin or a low grade intoxication. The lymphocytosis with few monocytes and eosinophiles indicates an attempt at immunohealing or repair by proliferation of connective tissue.

Briefly a low white cell count, neutropenia or, low neutrophilia, lymphocytosis, a few monocytes and eosinophiles, total shift cells under 35, and a slight stab left shift signify a low grade chronic intoxication of fibrotic character, and with typical clinical symptoms, points to chronic fibrous appendicitis. It is placed as appendicitis of the first degree because it is probably the least active and the most chronic form of appendicitis, if this fibrotic state can be called appendicitis.

Differentiation of first and second degree appendicitis lies chiefly in the white cell count, which most commonly approximates 6,000 in the first degree and 12,000 in the second degree process, other items of the hemogram being constant for both these degrees.

Second Degree Appendicitis.—A hemogram of this degree covers a group of pathologic conditions commonly classified as chronic inflammation of the

Number of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T normals
1	4400	1/7	16	1	0	0	30	28	58	0	5	0	36	41	1	0	0	00	00	5A
2	5900	1/20	6	2/5	0	0	20	50	70	0	0	0	20	20	10	0	0	00	00	5A
3	5950	1/14	8	1/2	0	0	14	28	42	0	0	0	48	48	6	0	4	00	00	5A
4	6900	1/14	8	1/2	0	0	22	42	64	0	16	0	20	36	0	0	0	00	00	5A
5	7000	1/27	5	1/3	0	0	12	39	51	3	27	0	12	42	1	3	3	00	00	5A
6	7150	1/13	9	3/5	0	0	27	42	69	0	3	0	27	30	1	0	0	00	00	5A
7	7800	1/36	4	1/4	0	0	10	45	55	0	2	0	35	37	8	0	0	00	00	5A
8	8050	1/11	8	1/2	0	0	22	38	60	0	10	0	14	24	14	2	0	00	00	5A
9	9400	1/10	10	2/3	0	0	24	34	58	0	8	0	14	22	14	6	0	00	00	5A
10	10900	1/28	4	1/4	0	0	12	42	54	0	4	0	30	34	10	2	0	00	00	5A

Chart 1.—First degree or chronic fibrous appendicitis, illustrating variation in white blood cell counts with uniformity of indices and other items of the hemogram.

appendix. Some of these processes, however, do not definitely show either the acute or chronic features of inflammation but are chiefly characterized by degeneration, atrophy or hypertrophy. Boyd, with some qualifications and discussion, describes most of these conditions, including fibrosis of the organ, under the heading of Chronic Appendicitis.

Some pathologists apply different names to various combinations of changes according to their individual views, e.g., chronic hyperplastic appendicitis which shows chiefly hypertrophy of the mucous glands, lymph follicles, muscles, or sympathetic nerve plexuses and sometimes distention of the lymph channels or infiltration of the tissues with lymphocytes; neurogenic appendicitis which is a term which may come into use with observation of hypertrophy of the nerve plexuses (Boyd); appendicosis or atrophic appendicitis, which is perhaps, more

a process of atrophy than inflammation; subacute appendicitis, a beginning acute exacerbation of chronic fibrous appendicitis or a late subsiding acute

No. of hemogram	Diagnosis	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
		7500	1/128	MI 1	ShI 1/6	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG	IB	T normals
1	Ch hyperplastic App, glands and follicles	16500	1/21	5	1/3	0	0	15	42	57	0	6	0	30	36	6	0	1	00	00	4
2	Ch hyperplastic App, glands and follicles	12000	1/8	16	1.0	0	0	30	30	60	0	0	0	36	36	4	0	0	00	00	4
3	Ch hyperplastic App,glands,follicles,lymph infil	11100	1/7	17	1.1	0	0	35	30	65	0	0	0	33	33	0	0	2	00	00	4
4	Neurogenic App (?) hypertrophy nerve plexuses	13000	1/9	16	1.0	0	0	35	37	72	0	0	0	22	22	4	2	0	00	00	4
5	Appendicosis	12400	1/10	16	1.0	0	0	30	36	66	3	6	0	24	33	1	0	0	00	00	4
6	Appendicosis	13000	1/22	5	1/3	0	0	18	45	63	3	3	0	18	24	9	3	1	00	00	4
7	Subacute App, Exacerb of ch	12400	1/17	8	1/2	0	0	25	50	75	0	0	0	20	20	5	0	0	00	00	4
8	Subacute App, subsiding acute	10800	1/10	12	3/4	0	0	34	40	74	0	6	0	2	8	14	2	2	00	00	4
9	Subacute App, subsiding,ac ex of ch	13700	1/14	10	2/3	0	0	20	35	55	0	20	0	15	35	10	0	0	00	00	4
10	Pyo-appendix	10000	1/25	5	1/3	0	0	18	57	75	0	2	0	9	11	14	0	0	00	00	4

Chart 2.—Second degree appendicitis, illustrating hemograms which are similar throughout but which represent subacute or chronic pathologic processes in the appendix, e.g., atrophy, hypertrophy, degeneration, inflammation, confined sterile pus or mucus and various combinations of these changes.

appendicitis; and hydro-appendix or pyo-appendix, when complete fibrotic stenosis of the proximal end of the appendix causes filling of the structure with mucus or pus which is probably sterile or contains an organism of very low virulence.

With vague symptoms and a history of recurrent attacks the hemogram of second degree appendicitis is quite constant for this group of conditions. The white blood cell count is moderately high and ranges from 10,000 to 15,000 per c.mm. The percentage of neutrophiles ranges from 50 to 75, total shift cells 15 to 35, lymphocytes 10 to 35, monocytes 1 to 15, eosinophiles 0 to 4, basophiles 0 to 3, lethal index $\frac{1}{25}$ to $\frac{1}{7}$, multiple index 5 to 17, Schilling index $\frac{1}{4}$ to 1.1, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 4.

Differentiation of second degree and third degree appendicitis: Confusion is not probable because of the acute symptoms and high white cell count and neutrophilia of the third degree or acute suppurative early gangrenous appendicitis, even though the low indices and low total shift cells may be like those of second degree appendicitis.

Number of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T normals
1	16300	1/38	3	1/5	0	0	16	76	92	2	4	0	2	8	0	0	0	00	00	1
2	18200	1/10	12	3/4	0	0	34	42	76	0	4	0	4	8	16	0	0	00	00	1
3	18500	1/20	5	1/3	0	0	22	62	84	0	5	0	8	13	3	0	0	00	00	1
4	19200	1/22	6	2/5	0	0	26	68	94	0	2	0	0	2	4	0	0	00	00	1
5	21000	1/22	6	2/5	0	0	25	68	93	0	3	0	4	7	0	0	0	00	00	1
6	22150	1/14	9	4/7	0	0	32	58	90	0	0	0	4	4	6	0	0	00	00	1
7	23000	1/18	8	1/2	0	0	26	54	80	0	6	0	6	12	2	4	2	00	00	1
8	25300	1/19	8	1/2	0	0	27	57	84	0	3	0	6	9	7	0	0	00	00	1
9	26200	1/10	10	2/3	0	0	36	54	90	0	0	0	4	4	6	0	0	00	00	1
10	31500	1/13	9	3/5	0	0	32	52	84	0	2	0	2	4	12	0	0	00	00	1

Chart. 3.—Third degree or acute suppurative early gangrenous appendicitis, illustrating high leucocytosis and neutrophilia but only a slight degenerative left shift. (Frozen threshold.)

Differentiation of second degree and fourth to eighth degrees of appendicitis is made easy by the higher white cell count, neutrophilia, wider shift, total shift cells over 35, and higher indices of the latter.

Third Degree or Acute Suppurative Early Gangrenous Appendicitis.—With typical symptoms of acute appendicitis this hemogram is remarkably constant. The white cell count is always high, approximating 18,000 per c.mm. with high neutrophilia. It is peculiar in that the left shift is always slight, with Schilling index a fraction, multiple index close to 16, and the total shift cells approximately 20. This inactivity of the maturation and release mechanism of the bone marrow, after becoming slightly depressed, we call a "frozen threshold." The area of gangrene may vary from the size of a pea to one-third of the appendix.

Toxin from this gangrenous area seems to stimulate the regenerative power of the bone marrow to produce large numbers of neutrophiles but at the same time possibly inhibits or "freezes" the threshold at a slightly depressed level so that only a few shift cells escape into the peripheral blood. To indicate the presence of confined, infected pus, 35 or more total shift cells are usually found, yet this hemogram, with as few as 15 shift cells, consistently indicates the condition to be an acute suppurative early gangrenous process when the clinical symptoms are typically those of an acute appendicitis.

The leucocyte count of third degree appendicitis ranges from 15,000 to 30,000 per c.mm., the neutrophiles from 75 to 95, total shift cells 15 to 35, lymphocytes 2 to 25, monocytes 1 to 15, eosinophiles 0 to 4, basophiles 0 to 2 per cent, lethal

Number of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T	normals
1	8000	1/5	25	1.6	0	0	42	25	67	0	15	0	12	27	6	0	0	00	00	2	
2	8400	1/5	24	1.5	0	0	50	20	70	0	12	0	8	20	6	4	0	00	00	2	
3	9800	1/4	36	2.3	0	0	53	23	76	0	0	0	23	23	1	0	0	00	00	2	
4	11100	1/8	16	1.0	0	0	36	36	72	0	0	0	16	16	12	0	0	00	00	2	
5	11600	1/7	19	1.2	0	0	40	35	75	0	8	0	10	18	7	0	0	00	00	2	
6	11900	1/6	20	1.3	0	0	40	30	70	0	22	0	0	22	4	2	2	00	00	2	
7	12700	1/5	25	1.6	0	0	42	25	67	0	5	0	20	25	0	8	0	00	00	2	
8	12800	1/8	19	1.2	0	3	35	32	70	0	10	0	7	17	12	1	0	00	00	2	
9	13000	1/3	40	2.5	0	0	50	20	70	0	2	0	28	30	0	0	0	00	00	2	
10	15300	1/6	19	1.2	0	0	42	33	75	0	6	3	6	15	8	2	0	00	00	2	

Chart 4.—Fourth degree or acute suppurative exacerbation of chronic appendicitis, illustrating variation in white blood cell counts and uniformity of indices and other items of the hemograms.

index $\frac{1}{40}$ to $\frac{1}{10}$, multiple index 3 to 16, Schilling index $\frac{1}{5}$ to 1, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 1.

Differentiation of third degree and the higher degrees of appendicitis is made chiefly on the "frozen threshold" or slight left shift, few shift cells and low indices, while the fourth to eighth degrees show a wide left shift and total shift cells usually over 40 per cent.

Fourth Degree or Acute Suppurative Exacerbation of Chronic Appendicitis.—With an acute attack in recurrent appendicitis this hemogram constantly shows a white cell count approximating 13,000, a neutrophilia close enough to 75 per cent to indicate an acute process, total shift cells usually 40 per cent or above indicating confined pus, while lymphocytes approximately 30 per cent and a few monocytes point to chronicity.

Fourth degree or acute suppurative exacerbation of chronic appendicitis shows a range of white blood cells from 7,000 to 15,000 per c.mm., neutrophilia 60 to 75, total shift cells 35 to 60, lymphocytes 20 to 40, monocytes 1 to 15, eosinophiles 1 to 10, basophiles 0 to 3 per cent, lethal index ⅛ to ⅓, multiple index 16 to 48, Schilling index 1 to 3, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2.

Fourth degree appendicitis is distinguished from first, second, and third degree processes by its higher indices and total shift cells being over 35, indicating pus. It is not confused with fifth, sixth, seventh, and eighth degrees chiefly because of its high lymphocytes, relatively low neutrophilia and low leucocyte count. In rare instances it may be confused with the sixth degree or acute suppurative appendicitis, not ruptured, when the latter is walled off by the omentum

No. of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T	normals
1	9100	1/4	35	2.2	0	0	47	25	72	0	3	0	25	28	0	0	0	00	00	2-4	
2	12300	1/5	28	1.8	0	0	36	20	56	0	6	0	10	16	28	0	0	00	00	2	
3	13700	1/6	20	1.3	0	0	43	33	76	0	5	0	19	24	0	0	0	00	00	2	
4	13850	1/5	22	1.4	0	3	45	33	81	0	0	0	15	15	4	0	0	00	00	2	
5	14200	1/3	40	2.5	0	0	50	20	70	0	16	0	0	16	12	1	1	00	00	2	
6	16100	1/6	19	1.2	0	0	38	30	68	2	6	0	6	14	18	0	0	00	00	2	
7	16550	1/5	22	1.4	0	3	36	27	66	3	9	0	12	24	10	0	0	00	00	2	
8	18100	1/6	17	1.1	0	0	45	39	84	0	6	0	0	6	10	0	0	00	00	2	
9	19300	1/5	24	1.5	0	0	48	32	80	0	6	0	10	16	2	2	0	00	00	2	
10	25100	1/5	20	1.3	0	0	37	28	65	0	0	0	28	28	7	0	0	00	00	2	

Chart 5.—Fifth degree or acute suppurative appendicitis, ruptured with walling off pus. Clinical prerequisite, mass in right lower quadrant and symptoms of acute appendicitis. This chart illustrated variation of leucocytosis and uniformity of indices and other items of the hemograms.

or mesentery which may induce an equally high lymphocytosis. Here the history aids in differentiation, as fourth degree is recurrent in type and sixth degree shows acute symptoms of a first attack.

Fifth Degree or Acute Suppurative Appendicitis, Ruptured, Mass in Right Lower Quadrant, Walling Off.—The prerequisites for this hemogramic diagnosis are a mass in the right lower quadrant with symptoms and history of ruptured appendix. The characteristic hemogramic features comprise a comparatively high percentage of lymphocytes and monocytes with a rather low neutrophilia. Total shift cells are over 35 per cent and the white blood cell count usually high but may be as low as 9,000 per c.mm. The higher the lymphocytes and monocytes the more advanced is the connective tissue proliferation in the walling off

process. The higher the neutrophilia and lower the lymphocytes and monocytes the less extensive is the walling off process. The percentage of total shift cells being over 35 points to confined pus.

In fifth degree appendicitis the total white cell count ranges from 10,000 to 30,000 per c.mm. In percentage the neutrophiles range from 60 to 90, total shift cells 35 to 60, lymphocytes 5 to 30, monocytes 0 to 30, eosinophiles 0 to 2, lethal index 1/8 to 1/3, multiple index 16 to 48, Schilling index 1 to 3, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 2, with an occasional Type 4 being seen.

Differentiation of fifth degree and other degrees of appendicitis.—The palpable mass in the right lower quadrant with history of first attack and symptoms of ruptured appendix rule out other forms of appendicitis. Without the symptoms of ruptured appendix but with first attack and palpable mass in the

No. of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T	normals
1	7300	1/4	27	1.7	0	0	48	27	75	0	0	0	15	15	10	0	0	00	00	4	
2	10400	1/4	28	1.8	0	0	54	30	84	0	2	0	8	10	6	0	0	00	00	2	
3	11200	1/3	36	2.2	0	0	60	26	86	0	4	0	10	14	0	0	0	00	00	2	
4	14300	1/7	17	1.1	0	0	48	42	90	0	0	0	3	3	7	0	0	00	00	2	
5	15200	1/5	28	1.8	2	2	54	32	90	0	10	0	0	10	0	0	0	00	00	2	
6	16000	1/4	35	2.2	0	0	55	25	80	0	5	0	8	13	7	0	0	00	00	2	
7	17100	1/5	24	1.5	0	0	57	38	95	0	0	0	3	3	2	0	0	00	00	2	
8	19200	1/4	28	1.8	0	0	52	28	80	0	0	0	16	16	4	0	0	00	00	2	
9	20000	1/6	20	1.3	0	0	47	35	82	2	0	0	8	10	8	0	0	00	00	2	
10	26200	1/7	17	1.1	0	0	48	44	92	0	0	0	8	8	0	0	0	00	00	2	

Chart 6.—Sixth degree or acute suppurative appendicitis, not ruptured, illustrating variation in leucocytosis with uniformity of indices and other parts of the hemograms. Total shift cells high but under sixty.

right quadrant this hemogram could indicate acute suppurative appendicitis, not ruptured but walled off by omentum or mesentery. If the history cannot be obtained, the mass in the right lower quadrant is not definite, and the pus is well walled off, inducing high lymphocytes, the fifth degree hemogram may be confused with that of the fourth degree or acute suppurative exacerbation of chronic appendicitis.

Sixth Degree or Acute Suppurative Appendicitis Not Ruptured.—With a history of first attack and acute symptoms of appendicitis this degree is characterized by high neutrophilia, lymphopenia, variable monocytes, total shift cells over 35 per cent, and white cell count usually high.

In sixth degree or acute suppurative appendicitis, not ruptured, the white blood cell count ranges from 7,000 to 30,000 per c.mm., neutrophiles 75 to 95, total

shift cells 35 to 60, lymphocytes 0 to 20, monocytes 0 to 10, eosinophiles 0 to 3, basophiles 0 per cent, lethal index 1/8 to 1/3, multiple index 16 to 48, Schilling index 1 to 3, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2.

Differentiation of this degree and seventh degree or acute suppurative appendicitis, ruptured, is based on the total shift cells being over 60 per cent in the latter, together with symptoms of ruptured appendix. Occasionally the sixth degree hemogram will fail, and the appendix be ruptured. In these cases the monocytes are high and range from 10 to 20 per cent, white blood cell count as high as 25,000, total shift cells will be between 50 and 60 per cent, neutrophiles approximately 80 per cent, and the multiple index will be above 25. The clinical symptoms and diagnosis, however, are those of ruptured appendix and peritonitis.

No. of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lympha	Intermediate lympha	Small lympha	Total lympha	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T normals
1	6000	1/5	28	1.8	0	0	60	33	93	0	0	0	3	3	4	0	0	00	00	2
2	11300	1/4	35	2.2	0	0	62	28	90	0	0	0	3	3	7	0	0	00	00	2
3	15600	1/5	27	1.7	0	0	60	35	95	0	0	0	5	5	0	0	0	00	00	2
4	17800	2/5	48	3.0	0	0	63	21	84	0	0	0	3	3	12	1	0	00	00	2
5	18400	1/5	32	2.0	2	4	54	32	92	0	6	0	2	8	0	0	0	00	00	2
6	20700	1/4	35	2.2	0	0	60	27	87	0	3	3	3	9	4	0	0	00	00	2
7	23200	1/4	30	1.9	0	0	62	32	94	0	0	0	2	2	4	0	0	00	00	2
8	28500	1/5	28	1.8	0	0	60	34	94	0	0	0	6	6	0	0	0	00	00	2
9	31200	1/5	29	1.8	0	4	56	34	94	0	2	0	2	4	2	0	0	00	00	2
10	33500	1/4	35	2.2	0	0	62	28	90	0	0	0	6	6	4	0	0	00	00	2

Chart 7.—Seventh degree or acute suppurative appendicitis, 75 per cent ruptured, 25 per cent ready to rupture, illustrating variation in leucocytosis with uniformity of indices and other items of the hemograms. Total shift cells 60 or above.

Seventh Degree or Acute Suppurative Appendicitis, Ruptured or Ready to Rupture.—With a history of first attack and symptoms of acute appendicitis the hemogram of this degree is characterized by high neutrophilia, lymphopenia, variable monocytes, practically no eosinophiles or basophiles, white blood cell count usually high and shift cells over 60 per cent.

Approximately 75 per cent of appendices showing this hemogram will be found to have ruptured, while 25 per cent will be ready to rupture. The latter show a small perforation or the wall to be so pervious to bacteria that a fibrinous peri-appendicitis and sometimes extensive gangrene have developed. The higher the total shift cells rise above 60 per cent the greater certainty there is that the appendix has ruptured. Nothing definite can be determined, in this regard, by the white blood cell count alone.

In the seventh degree or acute suppurative appendicitis, ruptured or ready to rupture, the white cell count ranges from 6,000 to 35,000 per c.mm., while all other features of the hemogram remain fairly constant for this condition. The neutrophiles range from 80 to 95, total shift cells 60 to 75, lymphocytes 0 to 15, monocytes 0 to 12, eosinophiles 0 to 3, basophiles 0 to 1 per cent, lethal index ⅕ to ½, multiple index 27 to 64, Schilling index 1.7 to 4, Freyfeld's toxin granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2.

Differentiation of the seventh degree or acute suppurative appendicitis, ruptured or ready to rupture, from sixth degree, or acute suppurative appendicitis not ruptured, lies in the total shift cells being over 60 per cent in the former. Seventh degree appendicitis is distinguished hemogramically from the eighth degree or acute suppurative appendicitis, ruptured with diffuse peri-

No. of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules %	Inclusion bodies %	Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T	normals
1	6100	1.8	262	16.4	2	10	70	5	87	2	4	0	2	8	5	0	0	100	20	3	
2	8000	9/10	140	7.5	0	2	73	10	85	0	10	0	3	13	2	0	0	00	00	2	
3	8750	1/2	67	4.2	4	14	45	15	78	1	11	1	8	21	1	0	0	00	00	2	
4	10300	9/10	125	7.8	2	4	72	10	88	0	2	4	6	12	0	0	0	00	00	2	
5	13900	12.0	1200	75.0	5	23	47	0	75	0	2	0	20	22	3	0	0	50	10	2	
6	14000	6.0	416	26.0	12	37	30	3	82	0	5	0	10	15	3	0	0	25	6	2	
7	15800	3.0	368	23.0	3	18	48	3	72	0	3	0	21	24	4	0	0	70	16	2	
8	16700	3.0	425	26.6	9	6	66	3	84	0	3	0	3	6	10	0	0	100	55	2	
9	21500	5/8	76	4.8	0	2	76	16	94	0	0	0	6	6	0	0	0	00	00	2	
10	37100	9/10	101	6.3	0	2	80	13	95	0	2	0	3	5	0	0	0	40	10	2	

Chart 8.—Eighth degree or acute suppurative appendicitis, ruptured with diffuse peritonitis. White blood cell counts vary. Other features of the hemograms are similar in that they show high neutrophilia, high total shift cells, wide qualitative left shifts, and very high indices which vary with extent of peritonitis or presence of septicemia.

tonitis, by the higher indices of the latter. Furthermore, diffuse peritonitis presents a much wider qualitative left shift, usually showing several myelocytes and juveniles in addition to stabs.

Eighth Degree or Acute Suppurative Appendicitis, Ruptured With Diffuse Peritonitis.—This hemogram is typical of diffuse peritonitis from any cause. The clinical history and symptoms of acute suppurative appendicitis with rupture of the appendix place it in this classification. The chief hemogramic features consist of high indices, high neutrophilia, and wide qualitative left shift with myelocytes and juveniles frequently high.

In the eighth degree or acute suppurative appendicitis, ruptured, with acute diffuse peritonitis, the white blood cell count ranges from 5,000 to 40,000 per c.mm., neutrophiles 75 to 100, total shift cells 75 to 100, lymphocytes 5 to 25,

Degree of appendicitis	Diagnosis	Thous WBC per cmm WBC 5-10	Total neutrophiles % TN 68	Total shift cells % TSC 4	Lethal index LI 1/128	Multiple index MI 1	Schilling index ShI 1/16	WBC per cmm WBC 7500	Lethal index LI 1/128	Multiple index MI 1	Schilling index ShI 1/16	Myelocytes M 0	Juveniles J 0	Stabs St 4	Segmenters Sg 64	Total neutrophiles TN 68	Lymphoblasts Lb 0	Large lymphs Intermediate lymphs L I 5 1	Small lymphs S 20	Total lymphs TL 26	Monocytes M 4	Eosinophiles E 2	Basophiles B 0	Type of hemogram T	
		Ranges										Typical hemograms													Normals
1°	Ch fibrous App, obliterative	4-10	40-70	10-35	1/40-1/7	4-16	1/4-1.0	5600	1/16	8	1/2	0	0	19	39	58	0	7	26	33	7	1	1	5A	
2°	Ch hyperplastic App, glands, follicles Neurogenic App, hypertrophy nerve plexuses Ch atrophic App, appendicosis Cat App, Pyo-App, Hydro-Appendix Subacute appendicitis	10-15	50-75	15-35	1/25-1/7	5-17	1/4-1.1	12500	1/15	12	3/4	0	0	26	40	66	0	5	21	26	6	1	1	4	
3°	Ac Supp early gangrenous App	15-30	75-95	15-35	1/40-1/20	3-17	1/5-1.1	18500	1/20	5	1/3	0	0	22	62	84	0	5	8	13	3	0	0	1	
4°	Ac Supp Exacerb of Ch App	7-15	60-75	35-60	1/8-1/3	16-48	1-3	13000	1/5	24	1.5	0	0	43	28	71	0	8	13	21	6	2	0	2	
5°	Ac Supp App, Rup, mass RLQ	10-30	60-90	35-60	1/8-1/3	16-48	1-3	15000	1/5	24	1.5	0	0	43	29	72	0	6	13	19	9	0	0	2	
6°	Ac Supp App, not ruptured	7-30	75-95	35-60	1/8-1/3	16-48	1-3	16000	1/5	26	1.6	0	0	52	33	85	0	2	8	10	5	0	0	2	
7°	Ac Supp App, Rup 75%, ready to Rup 25%	6-35	80-95	60-75	1/5-1/2	27-64	1.7-4	20000	1/4	33	2.0	0	0	61	30	91	0	1	4	5	4	0	0	2	
8°	Ac Supp App, Rup, diffuse peritonitis	5-40	75-100	75-100	1/2-20	64-1600	4-100	25000	7/8	152	9.5	4	12	60	8	84	0	5	8	13	3	0	0	2-3	

Chart 9.—Illustrating eight degrees of appendicitis by a typical hemogram of each degree together with its ranges in white blood cell counts, neutrophiles, total shift cells, lethal, multiple and Schilling indices.

monocytes 0 to 10, eosinophiles 0 to 2, basophiles 0 per cent, lethal index ½ to 20, multiple index 64 to 1,600, Schilling index 4 to 100, Freyfeld's toxic granules 0 to 100, Doehle's inclusion bodies 0 to 50, and the hemogram is Type 2 with high leucocytosis or Type 3 with leucopenia.

Differentiation of the eighth degree of appendicitis depends upon its higher indices and wider qualitative left shift, the history and clinical symptoms.

NECROSIS OF THE APPENDIX

A case is illustrated by Chart 10 in which the hemogramic diagnosis of acute suppurative appendicitis was not substantiated. Grossly the appendix appeared to be normal but histologic examination showed extensive necrosis with feces "burrowing" between the walls of the organ. This pathology was apparently responsible for the hemogramic picture and clinical symptoms since the hemogram became normal and the symptoms rapidly disappeared after appendectomy.

No. of hemogram	Date Aug. 1934	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram	Time	Days
No	D	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	SG 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T	T1	D
ShI																							
1	19	15100	4/5	102	6.4	0	0	64	10	74	0	0	0	23	23	3	0	0	00	00	2	Pre-op	
2	21	20700	2/5	48	3.0	0	2	63	18	73	0	5	0	13	18	9	0	0	00	00	2	Post-op	2
3	22	8400	1/5	28	1.8	0	2	43	25	70	0	13	0	7	20	10	0	0	00	00	4	Post-op	3
4	23	9300	1/5	24	1.5	0	0	43	28	71	0	2	0	27	29	0	0	0	00	00	4	Post-op	4
5	24	9600	1/9	10	2/3	0	0	28	33	61	0	0	0	30	30	9	0	0	00	00	4	Post-op	5

Chart 10.

COMMENT

Uniformity in results and interpretation obtained by various workers in Schilling hemograms depends upon acceptance of certain fundamental standards. We have utilized the following methods in this work:

1. Thin smears on slides.

2. Wright's stain with buffer solution.

3. Nomenclature: myelocytes, juveniles, stabs, and segmenters for the four types of neutrophiles, and "shift cells" when referring to myelocytes, juveniles, and stabs as a group exclusive of the segmenters.

4. Two points are sometimes confusing. The cytoplasm of the myelocyte is most frequently yellow and granular like that of the juvenile, stab, and segmenter (Pappenheim). It is light blue (Schilling) or colorless only in severe intoxications when, as in the other neutrophiles, the yellow neutrophilic granules are lost and the cell has become a toxic agranulocyte.

Schilling, in the *Blood Picture*, page 127, describes the nucleus of the segmenter (segment-nucleur, neutrophile leucocyte) in these words, "usually has 2 to 5 irregular segments which are connected in a chain of *fine threads.*" Cooke is frequently credited with having contributed this distinguishing feature. However, at least one thread of chromatin connecting two segments or a clear open space between two segments should be found to warrent classifying a neutrophile cell as a segmenter.

It is suggested that the study and interpretation of changes in the number, morphology, and combinations of the peripheral white blood cells, in single and sequential hemograms, give definite information regarding the character and extent of the underlying general pathology and serve as a valuable guide in determination of the special pathologic process. This method is not simply a different one of doing a "white and differential" but constitutes a new technic for the study of infectious diseases from the standpoint of diagnosis, prognosis and treatment.

From the study of the blood picture we recognize eight degrees of appendicitis with fairly characteristic hemograms for each. Occasionally the hemogramic diagnosis is not consistent with the pathologic condition of the appendix, as illustrated by the shiftogram of a case of necrosis of the appendix. That the apparently normal appendix, in this instance, was responsible for the preoperative clinical and hemogramic diagnosis was proved by the prompt postoperative right shift to recovery, subsidence of symptoms and by examination of the sections.

These hemograms are presented, not as definitely diagnostic, but as a valuable aid to diagnosis that can be supplied by the clinical laboratory. The limitations of the simple "white cell count and differential," in this respect, have long been recognized.

The term "degree" is used for comparison with the work of Kohl and Schilling. We do not, in any way, suggest the substitution of "degree" for the conventional terms now used for the various inflammatory states of the appendix.

The successful use of the Schilling hemogram is, in this field as in others, a matter of practice and experience. An effort should be made in every case to check the preoperative diagnosis suggested by the hemogram with the histopathologic diagnosis. This is not so essential when gross examination shows the appendix to be distended with pus, gangrenous or ruptured. Many such findings in our group were not checked by microscopic diagnosis.

If the hemogram is carefully studied, in the light of clinical data, accurate diagnosis may be made in about 90 per cent of cases. This refers to the "degree" of appendicitis; no attempt has been made in this paper to differentiate appendicitis from other pathologic processes.

SUMMARY

We have outlined a laboratory method for the diagnosis of different kinds of appendicitis by a modification of Kohl's method, based on Schilling's original work. Hemography is briefly discussed. Eight degrees of appendicitis are described hemogramically and differences pointed out. The diagnostic and prognostic values of the method are discussed.

CONCLUSIONS

1. A more accurate diagnosis of appendicitis can be made by a modified Schilling hemogram than by the use of any other form of white blood cell study. The history and clinical symptoms are, of course, to be considered in any event.

2. It is possible, by hemogram, history and symptoms to distinguish between eight degrees of inflammation or pathologic states of the appendix.

3. Limited white cell differential counts tend to discourage investigation of the blood picture in its relationship to infectious diseases and may close the door to much valuable information.

We express our appreciation to Dr. William Dameshek, Beth Israel Hospital, Boston, for many valuable suggestions. We are deeply grateful to the chiefs of the various wards of the Philadelphia General Hospital for their cooperation and permission to use their cases.

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THE EFFECT OF OBSTRUCTIVE JAUNDICE ON THE BLOOD PLATELETS OF THE RABBIT*

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SINCE Greig Smith[1] mentioned the danger of postoperative hemorrhage in patients with obstructive jaundice, abnormality of several of the elements of the coagulation system has been thought of as a possible explanation of this hemorrhagic tendency. Koechig[2] found a slight reduction in serum calcium. Kirk and King,[3] and Emerson[4] confirmed these findings, but demonstrated in addition even a relatively greater decrease in diffusible calcium. Walters and Bowler,[5] Snell, Greene, and Rowntree,[6] and Zimmerman[7] observed no decrease in total serum calcium. Gunther and Greenberg[8] demonstrated a decrease in total serum albumin, causing no decrease in the active or diffusible calcium.

Fibrinogen was found to be maintained at high levels in biliary obstruction experimentally by Moss[9] and clinically by Gram.[10]

Howell[11] has postulated that an excess of heparin may be the cause of the delay in clotting. Jones and Minot[12] found no decrease of the blood platelets in a case of catarrhal jaundice, but believed that there was a gradual quantitative increase as the depth of the jaundice decreased. Our own interest in the problem of the hemorrhagic tendency associated with jaundice arose in combination with a case of thrombocytopenic purpura which we had opportunity to study both clinically and at autopsy.

The patient, a white male, aged thirteen, had a clinical history of thrombocytopenic purpura with hemorrhages into the skin and mucous membranes of three weeks' duration. There was slight jaundice that had steadily been decreasing, and the blood showed an indirect van den Bergh reaction. The blood findings were: Hb. 8 gm.; R.B.C. 2,500,000; W.B.C. 7,500; differential, great predominance of polymorphonuclear cells; platelets 5,000. Autopsy confirmed the clinical impressions of hemorrhages into the skin, mucous membranes, and digestive tract. There were also hemorrhages into the pleurae and the kidney pelves. The liver showed biliary cirrhosis and marked jaundice. The retroperitoneal and mesenteric lymph node sinuses were filled with blood; the lungs showed an interstitial pneumonia.

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These findings caused us to wonder if jaundice such as obtained in this case might not be responsible for low platelet counts and hence an hemorrhagic tendency. We have not found in our reviews of the subject any continuous experimental or clinical observations on blood platelets during the course of an obstructive jaundice, and in view of this fact we have undertaken such a study.

MATERIALS AND METHODS

Rabbits were chosen as experimental animals because of the ease of taking blood samples from the ear and the convenient size of the animal. Adult female animals of three to six pounds weight were used, which were so far as could be ascertained, free from disease. The criteria used for determining the condition of the animals were: (1) condition of fur and general appearance; (2) appearance of viscera at laparotomy; (3) blood picture;* and (4) temperature.†

The diluting fluid used for the counting of platelets and erythrocytes was that of Rees and Ecker.[14]

Turck's solution was used as a diluting fluid in counting leucocytes.

Smears were stained with stock Wilson stain.

Blood samples were taken from the marginal vein of the ear at the same time each day to eliminate physiological variations of counts.[18, 20]

Platelets were counted by the direct method, using a $\frac{1}{100}$ dilution. The maximum error of this method, reckoning from simultaneous counts in practiced hands, is about 10 per cent. The success of the method is directly proportional to the lack of injury to the vessel, to the freeness of the blood flow, and to the rapidity of the dilution. The erythrocytes were counted directly from the same preparation. The leucocytes were counted directly in a $\frac{1}{20}$ dilution of Turck's solution. Hemoglobin calculations were determined by Sahli's method.

Smears were taken by the cover slip method, and stained with Wilson stain.

Drop ether anesthesia was used on all animals and all incisions were treated with collodion dressings. After operation animals were kept as quiet as possible for twenty-four hours and given only a little water. They were then fed routinely.

EXPERIMENT I

After being kept for one week under observation the animals were subjected to laparotomy and the common bile duct doubly ligated as it entered the duodenum. After this they were returned to their cages, where they received the regulation postoperative care. Daily postoperative records of

*The normal ranges of the blood elements were considered to be: Platelets, 250,000-750,000 per c. mm. (13, 14); physiologic variation 30 per cent; R.B.C. 4,500,000-6,250,000 per c. mm. (15); physiologic variation 15 per cent.

Leucocytes: 5,000-14,000 per c. mm. (16), Physiologic variation 40 per cent.

Differential (17):

Polymorphonuclears	43.4% (30-50%)
Lymphocytes	41.8% (30-50%)
Monocytes and transitionals	9.0% (2-16%)
Eosinophiles	2.0% (0.3- 5%)
Basophiles	4.3% (2- 8%)

†The normal temperature range was considered 38.3-40.8° C. (18).

the blood counts, temperatures, and clinical impressions were made during a period of observation ranging from fourteen to thirty-three days. The accompanying graphic representation of the blood findings (Group I) shows the effect of this procedure on the blood of six rabbits which had lived without complications sufficiently long to be considered typical.

Autopsies were done on each animal as soon after death as possible and the tissues fixed in Zenker's solution for microscopic study. A typical autopsy protocol is as follows:

Gross and Microscopic.—The animal appeared emaciated and yellow. The sclerae were intensely jaundiced. The peritoneum was shiny and glistening. The peritoneal cavity contained about 15 c.c. of free fluid. The omentum was adherent to the under surface of the liver. The liver, smaller than normal, was colored a yellowish brown. The portal fields were distinctly outlined. The gallbladder and ducts were extremely dilated, and were filled with small biliary calculi in dark green fluid. Occasionally these structures were found

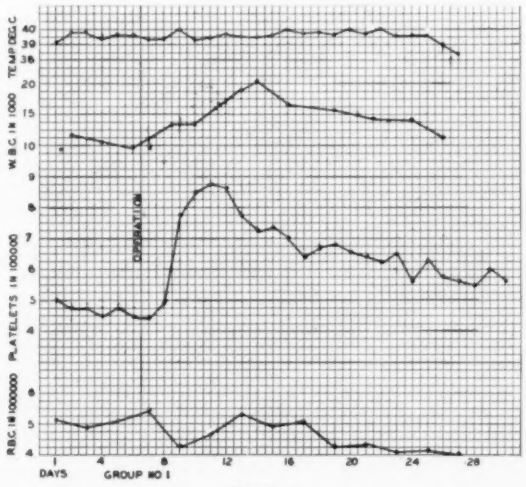

Chart 1.

to be filled with white bile. The spleen was greatly enlarged and was soft and spongy to touch. The capsule was very tight. A cross-section showed it to be engorged with blood. The malpighian bodies were easily seen.

The lower lobes of the lungs showed some consolidation which appeared to be bronchopneumonia.

Femur bone marrow presented a very great hyperplasia of the red cell forming element, and probably also hyperplasia of the white cell progenitors.

The kidneys and heart appeared normal.

Liver: The capsule was thickened in some places. The fibrous tissue around the periportal and portal areas was increased, more in some places than in others. These areas were filled with proliferating bile ducts, solitary liver cells, occasional small arteries, veins, and lymphatics. The area containing the liver cells also showed an occasional bile duct that was "sprouting" out. Areas of necrosis resembling infarctions of the parenchyma were sometimes found. The liver cells themselves were vacuolated. The nuclei showed no signs of degeneration, with the exception of those in the areas of necrosis. The liver cells did not contain large amounts of glycogen; the sinusoids were patent.

Kidneys: The capsule was normal. The capillary loops of the glomeruli were engorged. The convoluted tubules were filled with hyaline and fibrinous casts; there were places in

which one area showed great enlargement of the lumen with flattening of the epithelium as if by internal pressure. Degenerative processes were evident in some of these cells. This widening of the lumen and flattening of the epithelium was also observed in the loops of Henle and in the collecting ducts, which likewise contained large numbers of hyaline and granular casts. There was some engorgement of the vessels of the kidney.

Spleen: The splenic capsule seemed thinned, and the trabeculae were wide apart. There was an increase in the amount of fibrous tissue that formed the reticulum of the spleen. The sinusoids were filled with red cells, although there was no place in which an actual hemorrhage into the reticulum was seen. The malpighian corpuscles showed a decrease in size and number in proportion to splenic substance.

Lungs: The lungs showed some atelectasis and compensatory emphysema. A bronchopneumonia which appeared to be becoming confluent was evident in the left lower lobe. The bronchi were well filled with polymorphonuclear cells, but no bacteria could be seen.

Bone Marrow: There was a decided hyperplasia of both the red cell forming element and the myeloid forming element, bearing out the gross impressions.

CONTROLS—EXPERIMENT I

In our experimental animals three conditions had been produced that might have affected the rate of production or longevity of the blood platelet: (1) Bile had been backed up into the blood; (2) operative trauma had been produced; and (3) bile had been excluded from the intestine. Since we were interested only in the effect of abnormal concentrations of bile in the blood on the platelet, controls were devised. Our first control, designed to show the effect of operative trauma, is summarized in Chart 2, designated Group 2. Our second control, designed to show the effect of exclusion of the bile from the intestine, is summarized in Chart 3, designated Group 3.

In our first control group, one set of animals was subjected to the same preliminary study as the animals in Group 1 of the experiment, after which a laparotomy was performed. The common duct was picked up, a silk ligature placed around it and loosely tied so as not to obstruct the duct. The wound was repaired, a protective coat of collodion applied to the incision, and the animal returned to its cage. The usual daily record of blood counts, temperatures and clinical impressions was kept until the animal was killed (by a blow on the back of the neck). The postoperative period of observation ranged between fifteen and thirty-eight days. Chart 2 of the blood findings (Group 2) shows the effects of this procedure on the elements of four animals which were considered typical.

A typical autopsy report follows:

Gross and Microscopic.—The animal seemed well nourished; the general appearance was good. The fur was shiny and well kept. Stitches could be found in a well-healed abdominal scar. The peritoneum was shiny and glistening. There were some adhesions of the omentum to the upper part of the duodenum.

The liver, biliary system, kidneys, lungs, spleen, and heart appeared normal.

The femur bone marrow showed a hyperplasia of the red cell forming element, and possibly of the white cell forming element also.

The liver, kidneys, and spleen, microscopically, were normal.

Lungs: There were some areas of atelectasis, and some of emphysema. Occasionally one saw a small area that appeared to be an interstitial reaction, occupying, however, only a very small part of the lung. The bronchi were open and showed no evidence of inflammatory changes in these areas.

Bone Marrow: There was a decided hyperplasia of the red cell forming element, also of the myeloid elements, bearing out the gross impressions.

In our second control group (Group 3) the animals were observed for one week during which preliminary studies were made as in our experiment, a laparotomy done, and the common bile duct ligated by double ligatures at its entrance into the duodenum. A bell-shaped glass cannula was fastened in the lumen of

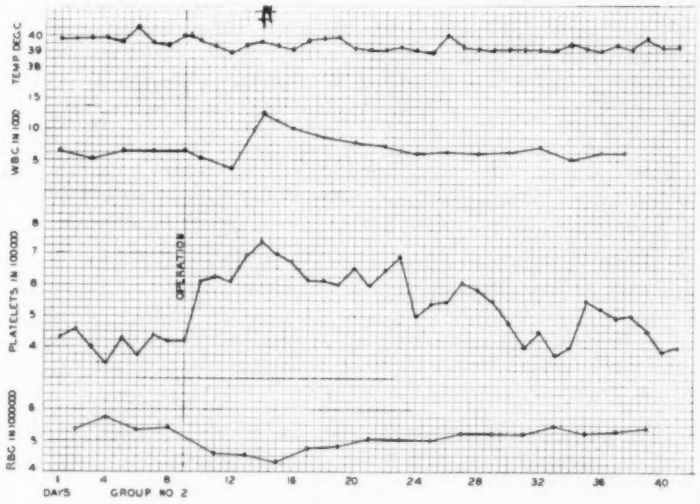

Chart 2.

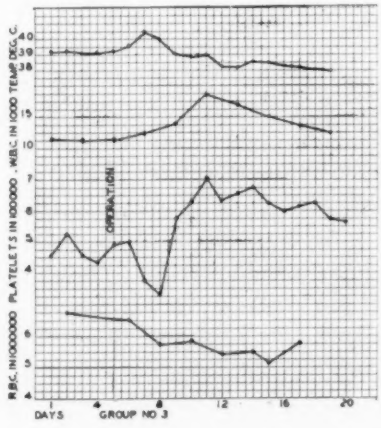

Chart 3.

the gallbladder with a purse-string. The bile was conveyed from the bladder cannula by means of a small rubber tube, which ran a short way through the subcutaneous tissue along the midline, to the outside of the body. The incision was coated with collodion, and the animals were returned to the cages. They were treated by the usual postoperative technic, care being taken to see that the bile was not ingested. Daily records of blood counts, temperatures, and clinical impressions were kept (Chart 3, Group 3).

A typical autopsy report follows:

Gross and Microscopic.—The animal was extremely emaciated, although the fur was still shiny. The bones protruded through the skin, and there seemed to be no subcutaneous fat. The muscle layers showed an encapsulated layer of leucocytes around the area through which the cannula ran.

The peritoneum was shiny and glistening.

The omentum was wrapped around the cannula, and was plastered up around the gallbladder and the under surface of the liver.

The liver was slightly smaller than normal. It was dark red in color and the portal fields well outlined. The gallbladder was securely wrapped around the cannula. A little fluid and a few small granules were present in the bladder and in the lumen of the cannula.

The spleen was slightly smaller than normal. The cut surface appeared normal, and the malpighian bodies stood out well.

The lungs showed a partial consolidation of the lower lobe of the left lung. The remainder of the left lung and the right lung did not show signs of infection.

The heart and kidneys were grossly normal.

The bone marrow of the femur appeared to show a hyperplasia of the red cell forming elements, and possibly of the white cell forming elements.

In our preliminary study of the animals used in our first experiment and in the above described controls of that experiment, it was shown that a postoperative rise in platelet count always occurred. In order to eliminate entirely this regularly occurring postoperative rise which was thought to be due most likely to trauma of operation, a second experiment was devised as follows:

A fourth set of animals was used, in which the bile duct was occluded by the following procedure without incurring the usual postoperative rise:

Laparotomy was performed and the common duct was surrounded by a loosely tied overhand knot of silver wire. The ends of this were brought through the body wall at the level of the tenth rib, and allowed to remain outside of the body, being first pulled to take up the slack. The abdomen was closed by the usual technic; the ends of the wire were wrapped around sponges and retained in position by means of a tape binder.

The usual postoperative care was observed; blood counts and temperatures were taken occasionally. After a lapse of two or three weeks, observations were made daily for one week before occluding the duct. The occlusion was accomplished by grasping the ends of the wires with straight clamps and pulling evenly on both sides until the knot was felt to be tight.

The blood findings gave no evidence of any constant or otherwise significant variation.

Complete occlusion of the duct was assured by autopsy findings, which were essentially those found in the animals in Group 1.

CONCLUSION

The production of a profound jaundice by the obstruction of the common bile duct produces no quantitative change in the blood platelets in rabbits that are not within the range of error of the method used.

It seems logical, therefore, to believe that the thrombocytopenia in the case observed was not due to the cholemia but coincident with it.

It follows that the explanation of any hemorrhagic tendency associated with jaundice must be sought elsewhere than in a diminution in the number of platelets in the circulating blood due to bile.

The author wishes to thank C. H. Evans, Jr., for aid in starting the problem; C. E. Isenhour whose aid and suggestions helped to finish it; and Drs. W. D. Forbus, D. H. Sprunt, and R. Baker whose constructive criticisms were invaluable.

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PRIMARY CARCINOMA OF THE LUNG*

A Review of Thirty Cases

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THE statement that primary carcinoma of the lung is on the increase has appeared frequently in the medical literature in the past decade. That statement combined with the fact that three patients with carcinoma of the lung were admitted to the medical service of the Wisconsin General Hospital within a few weeks of each other stimulated interest in this subject and led to the following survey.

At the Wisconsin General Hospital during the years 1925 to 1935, the diagnosis of primary carcinoma of the lung was made in 37 cases. In 30 cases the diagnosis was confirmed; (1) by postmortem examination in 25 cases, (2) by positive biopsy material from bronchoscopic examination in 2, (3) by bronchoscopic examination which revealed an obstructing tumor from which biopsy material could not be taken in two cases, (4) by positive x-ray evidence of a pulmonary neoplasm with concurrent metastases in ilium and femur in one case. Of the other 7 cases the diagnosis could not be confirmed; (1) four died, still having pulmonary signs and symptoms but no postmortem examination was performed and no biopsy had confirmed the impression of carcinoma of the lungs, (2) one died and postmortem examination revealed an adenocarcinoma of the pancreas with metastases to the lung, (3) one in whom the differential diagnoses included pulmonary tuberculosis was sent to a sanatorium for observation and is reported to be greatly improved (both clinically and to x-ray) making carcinoma improbable, (4) one is still alive, but there is no available material for biopsy, and bronchoscopic examination has failed to reveal any lesion although the x-ray findings are very suggestive. Therefore these 7 cases are not included in the survey.

Of the 30 cases reviewed the incidence by years reveals a striking similarity to the reports of others in that there has been a decided increase in the last five years.

YEARS	MALES	FEMALES	TOTAL
1925	0	1	1
1926	0	0	0
1927	0	0	0
1928	1	1	2
1929	2	0	2
1930	3	1	4
1931	2	2	4
1932	2	1	3
1933	3	0	3
1934	4	5	9
1935*	1	1	2
	18	12	30

*To June 1, 1935.

*From the Division of Medicine, University of Wisconsin.
Received for publication, July 6, 1935.

Many theories have been advanced to explain this increase but none has been definitely established.

Eighteen of the 30 cases were males and 12 females, giving a sex incidence of 3 to 2. This proportion is unusual since the incidence as recorded by others is usually much greater in the male. Vinson[1] reports a ratio of 4 to 1, Hruby and Sweany[2] 3 to 1, Olson[3] 4.5 to 1. The average age of the males in this series was 55.8 years and that of the females 44.8 years. These figures correspond fairly well with those recorded elsewhere. Hruby and Sweany[2] found an average age of 49.9 years, Olson[3] 53.7 years, and Bonner[4] concluded that the incidence was greatest in the sixth decade.

The past medical history of the cases in this series is significant in view of the work done by Askanazy (quoted by Hruby[2]) who found a peculiar regeneration of the bronchial epithelium after influenza and raised the question as to whether this might be a precursor of carcinoma. Although only 15 of the patients gave any history of antecedent pulmonary disease, 13 of these had had influenza and 8 reported a previous pneumonia or pleurisy.

The family histories of these subjects were unusually free from carcinoma. A brother of one patient had died of carcinoma of the pancreas. Otherwise there was no history of malignancy.

The occupations of these patients also appeared to be of little significance. The distribution of indoor and outdoor work was similar to that of the hospital admissions as a whole and the number engaged in occupations with special hazards was no greater than for the unselected cases.

Housewives	8	Not recorded	1
Laborers	4	Lumberjack	1
Farmers	3	Coal miner	1*
Teachers	3	Locomotive fireman	1*
Storekeepers	3	Textile worker	1
Metal workers	3	Electrician	1

*No suggestive history or physical and x-ray evidence of silicosis.

Since cough and chest pain are generally considered the two outstanding symptoms of carcinoma of the lung, it is interesting to note that of these 30 cases only 4 offered cough as the chief complaint and only 3, pain in the chest. These symptoms were present at some time in the course of the disease in the majority of the cases, however, only 3 being entirely free from cough, and 5 from pain. An appreciable weight loss was noted in 17 cases.

A survey of the signs reveals a striking incidence of fever. Twenty-five patients had a temperature of 100° or more at some time during the illness. Hemoptysis was reported in 8 cases, giving a low percentage as compared to other series of cases (Vinson[1] finding 43 out of 71 cases). The physical signs on examination of the chest varied with the location and character of the primary lesion and the associated conditions such as effusion, abscess, bronchiectasis, and atelectasis. In 3 cases the correct diagnosis was suggested primarily by definite signs of bronchial obstruction and in 3 others the signs were directional but not conclusive.

In 9 of the 30 cases bronchoscopic examination was made but revealed a neoplasm in only 4 instances. This low percentage of confirmatory findings is even more striking when one considers that of the 7 cases not included in this

study (of which 6 were very probably carcinoma of the lung) bronchoscopy was negative without exceptions. These results are very disappointing in view of the high percentage of positive findings in some series of cases such as Vinson's.[1] He found positive evidence in 70 of 71 patients examined.

An x-ray of the chest was taken in all but 2 patients. In the latter there had been nothing to suggest any pulmonary pathology. The x-rays revealed the following:

Definite x-ray evidence of carcinoma of the lung	7
Presumptive evidence of carcinoma of the lung	4
Other pulmonary pathology (effusion, atelectasis, etc.)	16
Negative finding	1

An anemia and leucocytosis were apparent in one-third and two-thirds of the cases respectively.

Anemia: Hemoglobin below 60 per cent in 10; average of entire group 63 per cent R.B.C.'s below 4.2 millions in 8; average of entire group 4.2 millions.
Leucocytosis: W.B.C.'s above 10,000 in 20 and above 20,000 in 6; average of entire group 18,000 neutrophiles above 85 per cent in 16; average 80 per cent.

A correct antemortem diagnosis was made in 18 of the 30 cases. An analysis of the antemortem diagnosis is as follows:

1. Antemortem diagnosis correct	18
2. Carcinoma of the lung mentioned in the differential	3
3. Diagnosis of pulmonary disease other than carcinoma	5
a. Antemortem diagnosis tuberculosis with effusion, 1	
b. Antemortem diagnosis empyema, 1	
c. Antemortem diagnosis bronchiectasis, 1	
d. Antemortem diagnosis chronic empyema, 1	
e. Antemortem diagnosis hypernephroma with metastases, 1	
4. Nothing antemortem to suggest primary carcinoma of the lung	4
a. Missed abortion with terminal pneumonia, 1	
b. Pernicious vomiting of pregnancy with liver atrophy, 1	
c. Carcinoma of prostate (incorrect), 1	
d. Benign hypertrophy of prostate, 1	

The primary lesion occurred almost equally in the right and left lung and was distributed as follows:

Right		Left	
Lung	3	Lung	3
Primary bronchus	3	Primary bronchus	3
Lower lobe	5	Upper bronchus	6
Lower bronchus	1	Hilum	1
Hilum	1	Pleura	1
	Bilateral fields	2	
	Bilateral hilum	1	

These figures compare rather closely with those of Olson,[3] who found 55 per cent of the squamous cell carcinomas in the left lung and 44 per cent in the right, an equal distribution of the adenocarcinoma, and 56 per cent of the undifferentiated on the left and 43 per cent on the right.

The microscopic report in the 25 postmortem cases showed the following types:

Bronchogenic	11
Adenocarcinoma	8
Alveolar	3
Unusual form	3

Weller's[5] statement that carcinoma of the lung rarely fails to metastasize is well illustrated in the series of cases under discussion. The following were noted:

Metastases

Liver	6	Pleura	2	Thyroid	1
Lymph nodes	5	Pancreas	2	Pericardium	1
Lung	4	Ribs	2	Heart	1
Adrenals	4	Ovaries	2	Skin	1
Kidneys	3	Brain	2*		

*Brain examined in only 2 cases.

As compared with other series there is apparently a very low incidence of metastases to the central nervous system and to bone. This is probably largely explained by the fact that the contents of the cranium were examined in only 2 cases (where the history and physical findings suggested intracranial involvement); and that search for metastases to bone (either by roentgenograms or necropsy) was not made in every instance.

SUMMARY

Thirty cases of primary carcinoma of the lung are reviewed and the significant findings include:

1. Marked increase in its incidence during the past five years
2. Common history of influenza
3. High incidence of fever
4. Infrequence of cough and chest pain as the chief complaint

In other details of clinical course, diagnosis, and necropsy findings, this series closely coincides with similar studies in the literature.

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THE ALLEGED VARIABILITIES OF KAHN AND WASSERMANN REACTIONS FROM METEOROLOGIC CHANGES*

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IN A recent article on serologic tests in syphilis, Hoverson and Petersen[1] conclude from their studies that results "indicate that in certain cases there occurred marked changes in the serology over short periods of time, measured in terms of days," and that "it is quite possible for a patient to have a four-plus Kahn or Wassermann one day, and a few days later to have negative reactions." They attempt to correlate the observed variations in degree of positivity of the Kahn and Wassermann tests with the barometric pressure and conclude further "that even such seemingly certain serologic reactions as those of syphilis are subject to the constant biologic tide as it is conditioned by the meteorologic environment."

Since their experiences and conclusions seemed contrary to ours and since as they state no similar study has appeared in the available literature, we have felt it worth while to examine our records for any similarly bizarre variabilities.

METHOD

While we have not followed the two- or three-day interval examination of blood serums, we have followed treated and untreated patients over a period of years and have made a practice of requesting second specimens to confirm our original findings on cases where a positive acknowledgment of syphilis was not given. Although in this group there was only a second test on the same individual, it is beyond the bounds of possibility that meteorologic conditions would always be identical when second specimens were taken. If meteorologic changes are really a factor in producing variations in serologic results, such variations must have occurred in many cases of our series, as the time intervals between the original specimens and the later ones were from several days to several months.

The serologic technic employed by us is the modification of the original Wassermann test recommended in Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. We employed two antigens, an alcoholic and a cholesterolized, purchased from the State Laboratory. Our results as compiled in this article are based on the cholesterolized results which, when they differed from the alcoholic, were higher with only one exception. The agreement between our original alcoholic result and that of the "retake" specimen was just as close as was the agreement between the original cholesterolized and the "retake" results cited in this article.

*From the Laboratories of the Life Extension Institute.
Received for publication, August 26, 1935.

RESULTS OF OUR STUDY

We have classified Hoverson and Petersen's Wassermann results which consisted of 98 tests in a series of eight cases, as to changes in successive reactions, i.e., a negative result to a doubtful or to a positive or vice versa. For this purpose we have classed all 2+ results, or stronger, as positive, ± and + as doubtful. These results are tabulated in Table I together with our results on a series of 421 cases of original and second or "retake" specimens.

TABLE I

WASSERMANN RESULTS

SERIES	NO. OF CASES	TOTAL TESTS PERFORMED	INSTANCES OF AGREEMENT BETWEEN DIRECTLY SUCCESSIVE TESTS		CHANGE FROM NEGATIVE TO DOUBTFUL OR VICE VERSA		CHANGE FROM NEGATIVE TO POSITIVE OR POSITIVE TO NEGATIVE		CHANGE FROM DOUBTFUL TO POSITIVE OR POSITIVE TO DOUBTFUL	
			NO.	%	NO.	%	NO.	%	NO.	%
Series of Hoverson and Petersen	8	103	44	45.83	22	22.9	21	21.9	9	9.37
Authors' series	421	842	384	91.21	16	3.8	2	0.48	19	4.51

In our series the great majority had never had syphilitic treatment. We repeated the Wassermann test on a second blood specimen taken from a few days to several months after the first bleeding. We found our second result exactly the same as the original in 310 instances (245 remained 4+, 20 remained 3+, 10 remained 2+, 11 remained +, 21 remained ± and 3 remained negative). In 74 instances the change of reaction (as 4+ to 3+, or ± to +) did not take the test out of the positive or doubtful group in which the original result was classed.

Although Hoverson and Petersen did not compare their corresponding Wassermann and Kahn reactions in their total of 98 tests on 8 individuals where comparison was possible, we have done so in Table II and have further compared them with a series of 1,714 cases previously reported by us.[2]

TABLE II

COMPARISON OF KAHN AND WASSERMANN REACTIONS

SERIES	CASES	CORRESPONDING TESTS PERFORMED	AGREEMENT KAHN AND WASSERMANN		WASSERMANN POSITIVE KAHN NEGATIVE—KAHN POSITIVE WASSERMANN NEGATIVE		WASSERMANN POSITIVE KAHN DOUBTFUL—KAHN POSITIVE WASSERMANN DOUBTFUL		WASSERMANN DOUBTFUL KAHN NEGATIVE—KAHN DOUBTFUL WASSERMANN NEGATIVE	
			NO.	%	NO.	%	NO.	%	NO.	%
Series of Hoverson and Petersen	8	98	48	48.98	28	28.57	18	18.37	4	4.08
Authors' series	1714	1714	1589	92.6	28	1.7	44	2.5	53	3.2

Their tests showed an agreement between positive Wassermann and positive Kahn reactions of 25.51 per cent, and a disagreement between negative Wassermann and positive Kahn of 28.57 per cent.

COMMENT

The extreme variations in serologic reactions for syphilis reported by Hoverson and Petersen and their wide divergencies between the results of Kahn and Wassermann tests on identical specimens are contrary to our own and to common experience. That meterologic changes could account for such inconsistencies is not supported by the results of our study. As noted in Table I our repetitions were in the majority of instances highly consistent with the original tests. If meteorologic variations are such an important factor as Hoverson and Petersen assert, supporting evidence should not be difficult to find in the vast literature which has accumulated on the subject of Wassermann and Kahn reactions.

We believe that Hoverson and Petersen must look elsewhere for a satisfactory explanation of their results.

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THE MODIFICATION OF THE HEMATOPOIETIC FUNCTION IN THE RABBIT BY CERTAIN CYCLIC COMPOUNDS*

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THE clinical literature which has accumulated around the subject of agranulocytic angina indicates that certain therapeutic agents, whose structures are fundamentally organic ring compounds, play an important rôle in the etiology of the disease. Amidopyrine is the most commonly reported etiologic agent,[1-3] but cases have been reported where the precipitation of the syndrome has been attributed to dinitrophenol,[4, 5] arsphenamine,[6] antipyrexol,[7] urea stibamine,[8] and nirvanol[9] among numerous other substances. The clinical evidence regarding the relationship between amidopyrine and agranulocytosis is sufficiently strong to leave no room for dubiety. Madison and Squier[1] described a patient who had recovered from a previous attack of agranulocytic angina and had shown a normal leucocyte level for a period of ten months. Within three hours of the administration of 5 gr. of amidopyrine, the patient suffered a chill and a recurrence of the acute symptoms: within twelve hours the granulocytes had disappeared from the circulation. Nor is this an isolated instance, for similar cases have been reported by Benjamin and Biederman,[10] and by Johnson.[11] Such evidence as this cannot be disregarded.

Unfortunately, however, the experimental demonstration of this relationship in laboratory animals has not been nearly as convincing: Madison and Squier administered allyl-isopropyl-barbituric acid together with amidopyrine to eighteen animals in relatively enormous doses. Only one animal in this series showed a diminution in the total number of leucocytes in the peripheral circulation and even this animal did not present a picture which resembled human agranulocytosis, for the granulopenia was accompanied by a marked diminution in the number of erythrocytes. Similarly, Zia and Forkner,[8] who had previously reported eight cases of agranulocytosis in a series of 72 patients who were being treated with urea stibamine or neostibosan, were unable to show any changes in the blood picture of rabbits that received large quantities of these drugs over a prolonged period of time. These authors, however, did show that the bone marrow of the treated animals became hyperplastic.

In this study the action of a group of cyclic compounds on the hematopoietic tissues was studied with the hope of throwing some light on the nature of the leucotoxic action. These substances included amidopyrine (phenyl-dimethyl-dimethylamino-pyrazalon), antipyrine (phenyl-dimethyl-pyrazalon), alpha-dinitrophenol, catechol and quinone. It was observed that the administration of

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large doses of amidopyrine or dinitrophenol, ranging from ten to forty times the normal human therapeutic dose, for prolonged periods of time, seemed to produce no change in the peripheral blood picture. An examination of the bone marrow of these animals, however, showed varying degrees of myeloid hyperplasia, areas of degeneration, and a definite suppression of maturation. It was felt that this hyperplasia constituted so serious a modification of the hematopoietic system that the change should manifest itself in the peripheral blood picture. Accordingly, a test was devised to examine the efficiency of the leucogenic system.

Under normal conditions, the leucopoietic apparatus of the rabbit is an organ in an extremely delicate state of equilibrium and the threshold for leucopoietic stimuli is very low. It has been shown[12] that even the intravenous injection of a small quantity of distilled water may be capable of affording such a stimulus. The effects of this stimulation are manifested in the peripheral circulation by a transient leucocytosis which is a true bone marrow reaction, for it is characterized not only by an increase in the total number of granulocytes in the peripheral circulation but also by a marked increase in the number of juvenile cells. This liberation of juvenile cells into the circulation is much more significant than the transient increase in the total number of circulating cells, for the ratio of juvenile cells to mature cells is a much more constant factor than the total number of cells in the peripheral circulation.

The more potent stimulating agents produce a more profound and a more consistent effect. Nucleic acid and sodium nucleinate are examples of such potent stimulating agents and the parenteral administration of either of these substances into a normal rabbit will invariably be followed by a marked increase in the percentage of juvenile cells in the peripheral circulation. Table I is an example of the reaction of a normal rabbit to the intramuscular injection of 5 mg./kilo of sodium nucleinate.

TABLE I

DATE	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					
					I	II	III	IV	V	WEIGHTED MEAN
9/20	6.2	75	9.2	28	23	33	29	12	3	2.39
9/21	6.2	75	8.1	30	22	33	31	11	3	2.40
9/22	6.4	75	10.4	28	22	31	30	12	5	2.47

Following the intramuscular injection of 5 mg./kg of sodium nucleinate, the following results were obtained:

HOURS AFTER INJECTION	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					
					I	II	III	IV	V	WEIGHTED MEAN
0	6.4	75	8.2	29	24	32	30	12	2	2.36
1	—	—	8.8	31	30	38	23	8	1	2.12
2	—	—	14.2	37	41	28	21	10	0	2.00
4	—	—	15.1	34	42	28	18	12	0	2.00
8	6.3	74	10.6	34	40	29	20	11	0	2.02
24	—	—	8.6	36	31	39	21	9	0	2.17
48	6.28	75	7.8	32	27	43	23	7	0	2.10
96	6.1	75	8.0	27	25	33	32	9	1	2.28
240	6.2	75	6.8	29	21	32	30	14	3	2.46

Amidopyrine.—A series of eight rabbits was examined and a normal nucleinate reaction established for each animal. Seven of the animals received 200 mg./kilo of amidopyrine daily (orally) and the remaining animal was used as a control for the group. Daily observations included a complete blood picture with a record of red cell count, white cell count, hemoglobin content, differential leucocyte count, and polynuclear count.[13] (This experimental procedure was followed for all the substances studied.) Animals were taken from the group at periodic intervals and killed in order to provide bone marrow specimens. The reaction to sodium nucleinate was observed at definite intervals during the course of the experiment. Table I also shows the normal reaction of an experimental animal to the intramuscular injection of 5 mg./kilo of sodium nucleinate prior to the administration of the amidopyrine.

When the normal blood picture had been reestablished this animal received 200 mg./kilo of amidopyrine daily. After the tenth day of this treatment a similar quantity of sodium nucleinate was again administered, but this time it failed to elicit the normal reaction as may be observed from an examination of Table II. At this time the control animal responded to the second injection of

TABLE II

DATE	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					
					I	II	III	IV	V	WEIGHTED MEAN
10/22	5.8	79	9.2	28	25	35	27	9	4	2.32
10/23	6.05	75	8.6	31	24	36	24	11	5	2.32
10/25	5.9	75	11.4	27	27	34	28	9	2	2.25

Following the intramuscular injection of 5 mg./kg of sodium nucleinate, the following results were obtained:

HOURS AFTER INJECTION	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					
					I	II	III	IV	V	WEIGHTED MEAN
0	6.1	74	8.2	27	28	37	27	8	2	2.25
1	—	—	6.8	26	28	36	26	8	4	2.30
2	—	—	7.2	27	26	38	25	9	2	2.23
4	—	—	7.6	28	27	33	27	10	3	2.29
8	—	—	8.6	30	26	34	28	9	3	2.31
24	6.3	71	8.4	29	25	36	24	11	4	2.33
48	6.0	73	7.6	30	29	32	25	12	2	2.26
96	6.05	73	6.8	27	26	35	27	9	3	2.28
240	5.85	76	9.4	29	24	34	28	10	4	2.36

sodium nucleinate in the same manner it responded to the original injection. The animal that had received the amidopyrine showed a perfectly normal blood picture at this time: erythrocytes, leucocytes, and differential leucocyte counts showed no aberration from the normal. An examination of the bone marrow, however, showed a marked degree of myeloid hyperplasia as may be observed by comparing the bone marrow of this animal (Fig. 2) with that of a normal animal (Fig. 1). The general appearance of the animal, as judged by behavior, weight, and feeding, was normal.

The second animal in the group received the intramuscular injection of sodium nucleinate on the fifteenth day, during the course of which time 3,000

mg./kilo of amidopyrine had been administered. No reaction was elicited and an examination of the bone marrow showed an even more marked degree of myeloid hyperplasia together with a definite suppression of maturation. In addition to the myeloid hyperplasia there was a marked increase in the number of megakaryocytes. This animal, in spite of the severe changes in the bone marrow, showed no change in the peripheral blood picture which could be brought out by the usual methods of examination (Fig. 3).

The next animal in this series failed to show a normal nucleinate reaction on the twenty-second day of treatment, but presented no abnormalities of the blood picture. Here the bone marrow showed focal areas of degeneration and necrosis which, on casual examination, seemed to be relegated to the myelogenous

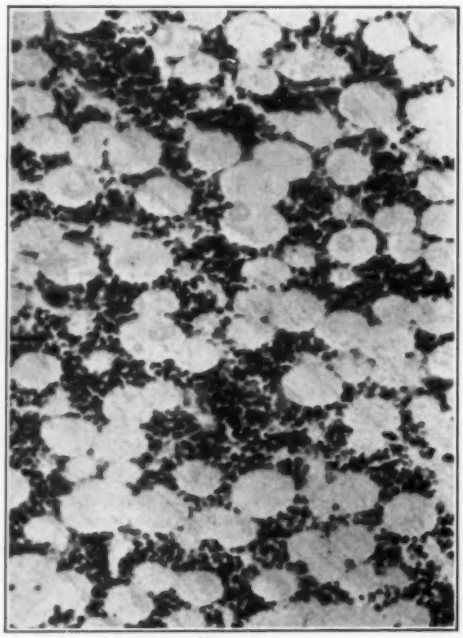

Fig. 1.

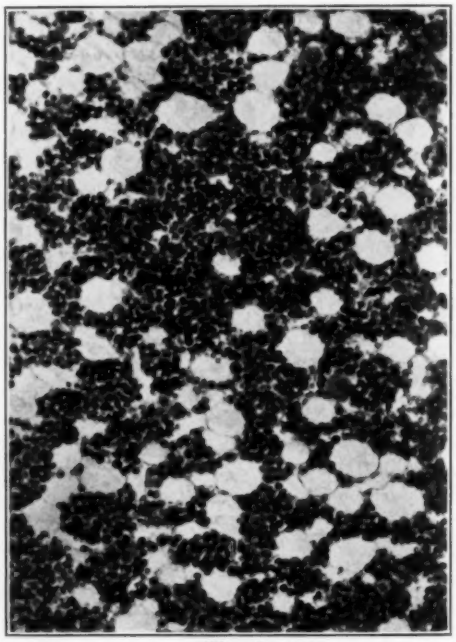

Fig. 2.

Fig. 1.—Femoral bone marrow. Normal rabbit.

Fig. 2.—Femoral bone marrow. Amidopyrine 200 mg. per kilo per day (orally). Tenth day. Marked myeloid hyperplasia.

elements. On closer examination, however, it soon became obvious that there was no specificity about the degeneration and that all the cellular elements were equally affected. The apparent selectivity which was observed may be explained when one realizes the fact that this bone marrow had undergone a massive myeloid hyperplasia and that the predominating cellular elements were cells of the myeloid series.

About the twenty-fifth day the animals of this series began to show an increasing lassitude and anorexia. Only portions of the daily ration were consumed and the animals became listless and inactive. About this time changes began to appear in the peripheral blood picture. These were characterized by

a fall in the erythrocyte level and a fall in the leucocyte level. Red cells fell as low as 3,900,000 and white cells as low as 3,200. The bone marrow at this

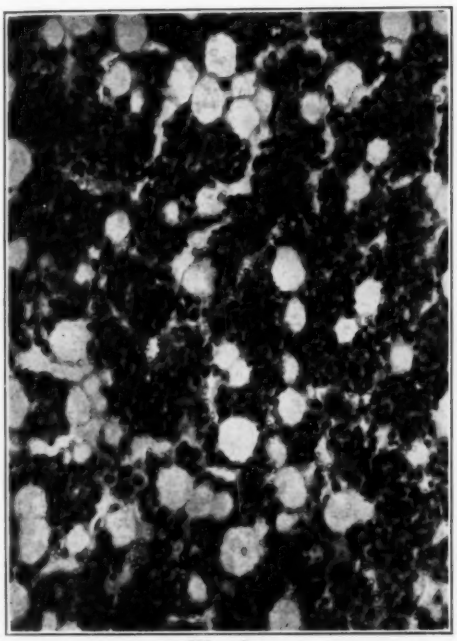

Fig. 3.

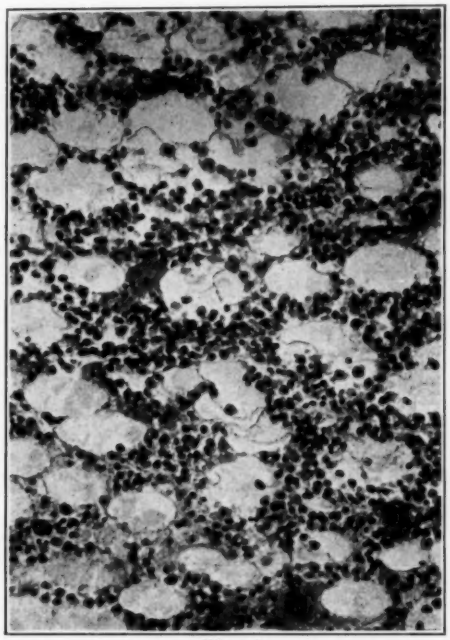

Fig. 4.

Fig. 3.—Femoral bone marrow. Amidopyrine 200 mg. per kilo per day (orally). Fifteenth day. Marked myeloid hyperplasia with suppression of maturation.

Fig. 4.—Femoral bone marrow. Amidopyrine 200 mg. per kilo per day (orally). Twenty-second day. Areas of necrotic degeneration superimposed on the hyperplastic marrow.

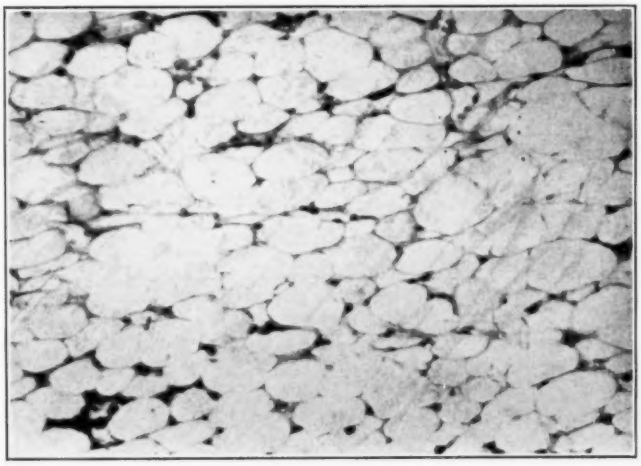

Fig. 5.—Femoral bone marrow. Amidopyrine 200 mg. per kilo per day (orally). Twenty-sixth day. Aplastic marrow.

time was almost completely aplastic (Fig. 5). Within two days of the appearance of these symptoms the animal died; death was invariably accompanied by an infection of the upper respiratory tract.

TABLE III

AMIDOPYRINE: 200 MG./KG/DAY (ORALLY)

RABBIT	DAY	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	NUCLEINATE REACTION
1	1	5.8	79	8.6	31	Positive
(Control)	3	5.9	79	9.7	33	
	5	5.9	77	10.2	29	
	7	6.1	79	11.2	33	
	9	6.0	80	9.4	31	
	11	6.0	80	8.7	32	Positive
	13	6.2	80	9.2	29	
	15	6.3	79	8.7	29	
	17	6 2	80	11.5	32	
	19	6.1	80	8.2	28	Positive
	21	6.1	80	8.6	27	
	23	6.1	79	10.4	29	
	25	6.1	79	9.8	30	
	27	5.9	80	8.8	30	
	29	5.9	80	7.6	30	Positive
	31	6.0	80	9.8	29	
2	1	6.5	83	11.4	36	
	3	6.3	82	9.8	36	
	5	6.3	82	8.4	36	
	7	6.4	82	10.7	35	
	9	6.3	83	9.4	36	
	10	6.3	83	12.5	36	Negative
3	1	6.8	79	14.5	34	
	3	6.8	79	10.2	34	
	5	6.8	81	12.8	32	
	7	6.4	79	14.2	31	
	9	6.5	80	10.8	34	
	11	6.2	80	11.6	33	
	13	6.4	80	9.8	33	
	15	6.0	80	11.2	33	Negative
4	1	6.25	76	11.2	33	
	3	6.15	74	8.6	33	
	5	6.15	74	7.8	33	
	7	6.2	73	10.4	30	
	9	6.35	74	8.4	32	
	11	6.1	73	10.0	32	
	13	6.15	73	10.8	32	
	15	6.2	73	9.4	31	
	17	6.25	73	9.6	32	
	19	6.0	74	8.5	32	
	21	6.0	72	11.5	31	
	22	5.9	72	10.2	32	Negative
5	1	5.8	79	10.3	29	
	3	5.9	79	8.6	32	
	5	5.8	77	9.8	31	
	7	6.1	79	10.4	29	
	9	6.0	80	8.2	28	
	11	6.35	81	7.4	29	
	13	5.9	74	8.2	27	
	15	6.0	75	7.8	29	
	17	6.0	75	6.8	28	
	19	5.9	72	7.2	28	
	21	5.2	70	6.2	26	
	23	4.8	68	3.6	20	
	25	4.2	65	3.8	19	Negative (died)
	27	3.9	59	3.2	17	

TABLE III—CONT'D

RABBIT	DAY	R.B.C. 10^6	Hb	W.B.C. 10^3	PERCENTAGE GRANULOCYTES	NUCLEINATE REACTION
6	1	6.4	77	16.4	24	
	3	6.45	78	14.2	26	
	5	6.4	77	15.4	25	
	7	6.25	77	16.0	24	
	9	6.4	76	12.5	27	
	11	6.4	77	14.0	27	
	13	6.3	77	15.0	26	
	15	6.0	74	14.2	25	
	17	6.25	75	10.6	22	
	19	6.1	74	9.8	21	
	21	5.9	73	8.5	20	
	23	5.65	70	6.8	18	
	25	5.20	68	7.2	18	Negative
	27	4.10	65	6.4	20	
7	1	6.7	76	8.2	26	
	3	6.75	76	9.6	26	
	5	6.8	75	7.3	26	
	7	6.6	76	8.4	25	
	9	6.7	76	9.2	24	
	11	6.7	75	11.3	27	
	13	6.8	74	9.8	26	
	15	6.8	75	8.1	25	
	17	6.4	75	8.0	24	
	19	6.0	69	7.6	21	
	21	5.2	68	7.2	21	
	23	5.0	68	6.8	19	
	25	4.8	64	4.2	19	Negative
8	1	6.2	68	5.4	25	
	3	6.3	68	6.8	25	
	5	6.2	68	16.4	28	(Died on tenth day)
	7	6.35	68	14.8	30	
	9	6.3	67	15.2	31	

Table III is an abridged protocol of the results obtained following the administration of amidopyrine. It should be pointed out, however, that the quantity of the drug that was administered each day represents about two-thirds of the minimal lethal dose and is equivalent to about 200 gr. of the drug for the normal human adult or approximately forty times the usual therapeutic dose.

Dinitrophenol.—The intramuscular administration of 20 mg./kilo/day of dinitrophenol to rabbits produced, save for a number of minor differences, the same changes in the hematopoietic system and in the blood picture as were produced by the administration of amidopyrine. For the sake of brevity, the detailed protocols are omitted. For the first two to five days after the administration of the dinitrophenol, the animals showed a slight but definite leucocytosis which was characterized by an increase in the percentage of juvenile granulocytes in the circulation. This leucocytosis, which was probably an expression of the general metabolic stimulation, returned to the normal level within a period of three to five days. The blood picture was otherwise unaffected for the first twenty-five days, after which both the erythrocyte level and the leucocyte level began to fall rather sharply. The nucleinate reaction be-

came negative after the twelfth day. When the dinitrophenol had been administered for about twenty-five days, and changes were beginning to be apparent in the blood picture, two of the animals developed sloughing necrotic ulcers in the gluteal region at the site of injection. Both of these animals died three days after the appearance of the ulcer.

Antipyrine.—From a qualitative point of view the action of antipyrine very closely simulated the action of amidopyrine. The fall in the total leucocyte count, however, was not as marked as the fall produced by the administration of either amidopyrine or dinitrophenol. The fall in the erythrocyte count appeared after the sixth day of administration, but was not as precipitous nor did it reach as low a level as the fall in either of the other two series. The nucleinate reaction in this series became negative after the fifteenth day.

Catechol, Quinone.—The action produced by both catechol and quinone was so erratic that the relatively small number of animals used provided no significant results. Doses which were tolerated produced no definite results, while the attempt to increase the dose level only produced symptoms of acute toxicity.

DISCUSSION

The disparity which exists between the clinical evidence as to the etiologic relationship of a group of drugs to agranulocytic angina and the experimental demonstration of this relationship on laboratory animals need not be regarded as directly contradictory. In human agranulocytosis, it is probable that the leucotoxic principle which is involved is not the drug itself, but some oxidation or conjugation product of the drug. In addition to this it is also probable that the leucotoxic action is not a toxic action in the ordinary meaning of the term, but rather a manifestation of an allergotoxic phenomenon with the granulocytes of the peripheral circulation acting as the shock organ. This must be true when one realizes the tremendous number of people who consume large quantities of these drugs without showing any untoward effects.

If the leucotoxic principle is a conjugation or oxidation product of the drug, then it is not surprising to find that experimental animals do not show this specific toxic action, for it is well known that the conjugation of an organic substance may vary from species to species. The classical example of such species variation is to be found in the handling of benzoic acid in the dog and in man. The human being conjugates this substance with glycine, while the dog conjugates it with glycuronic acid. The quantity of glycuronic acid which is available is much greater than the quantity of glycine; therefore, it is not surprising to find that the dog is capable of handling many times the quantity of benzoic acid a man can handle. Variations in the handling of cyclic organic compounds may occur among different individuals of the same species and even in the same individual at different times. This is clearly demonstrated to be the case with cinchopen, for Skorczewski and Sohn[14] have demonstrated that when this substance is administered to a normal individual the greater part of it is converted into oxycinchophen and is excreted in this form. As the length of

time increases during which the drug is being administered, the quantity of oxycinchophen which may be recovered diminishes. After a variable period of time the oxycinchophen disappears entirely, and the cinchophen is excreted in some other form.

From the experimental findings in this study, it is obvious that the administration of large quantities of amidopyrine, antipyrine, or dinitrophenol is capable of producing deleterious changes in the hematopoietic system. A striking characteristic of these changes is the fact that the bone marrow may undergo serious modification without producing any alteration in the peripheral blood picture which is discernible by the ordinary methods of examination. The application of a test of the functional activity of the leucopoietic system, however, shows that it has been definitely impaired at a time when no obvious changes are present in the blood picture.

The changes which are produced by the experimental administration of these drugs differ markedly from those seen in human agranulocytic angina and more closely approximate those found in cases of phenylhydrazine poisoning. In addition to this fact, the quantity of the drug which is required to produce these changes is far in excess of the dose range encountered in therapeutics. However, it is shown that the administration of these substances is capable of modifying the hematopoietic activity of experimental animals.

SUMMARY

1. A test is described for estimating the efficiency of the leucopoietic function in the rabbit based on the reaction to the parenteral administration of sodium nucleinate.

2. The continued administration of large quantities of amidopyrine, antipyrine, or dinitrophenol impairs the functional activity of the leucopoietic system of the rabbit.

3. This impairment of functional activity is associated with a series of morphologic changes in the bone marrow. These changes are successively: (a) myeloid hyperplasia, (b) myeloid hyperplasia with suppression of maturation, (c) focal degeneration and necrosis, and (d) aplasia.

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SODIUM CITRATE—A SPIROCHETICIDE*

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A TWO per cent solution of sodium citrate is frequently used in the laboratory to prevent coagulation of cover slip preparations of blood and exudates while the specimens are being examined. Its effect upon the bacterial flora of the specimens is not recorded. Commonly used in blood transfusions and internally as an alkalizing agent, clinically it is not generally considered to possess bactericidal properties.

An observation of the drug's action upon mouth spirochetes may be of interest to those concerned with the prophylaxis and therapy of spirochetal diseases.

A rather thick cover glass specimen from a laryngeal lesion of Vincent's angina was prepared for dark-field examination. It contained many pus cells and a bacterial flora consisting mainly of myriads of Vincent's organisms. The addition of a drop of a 2 per cent sodium citrate solution caused immediate cessation of movement of the spirochetes and apparently eventual dissolution. Repeated examinations gave the same results.

Later, through the courtesy of Dr. Hugh Lokey, whose patient was being examined, the same solution was applied to the surface of the ulcer from which the specimen had been taken. Ten minutes later no Vincent's organisms were found. None were present the next day or the day following, when the patient was dismissed. Should this drug find a place in the local or intravenous treatment of spirochetal infections, it might replace much more expensive and harmful therapy.

* Received for publication, July 20, 1935.

LABORATORY METHODS

INTESTINAL ILLUMINATOR*

A Device for Detecting Intestinal Lesions in Postmortem Specimens by Reflected and Transmitted Light

Joseph Felsen, M.D., New York, N. Y.

THE device pictured is essentially a rectangular box with the bottom and one long side removed and the top replaced by ground or opal glass. Illumination by reflected light is obtained by means of a 60- or 75-watt bulb situated above the glass plate in a built-up portion of the intact long side (*A*). Illumina-

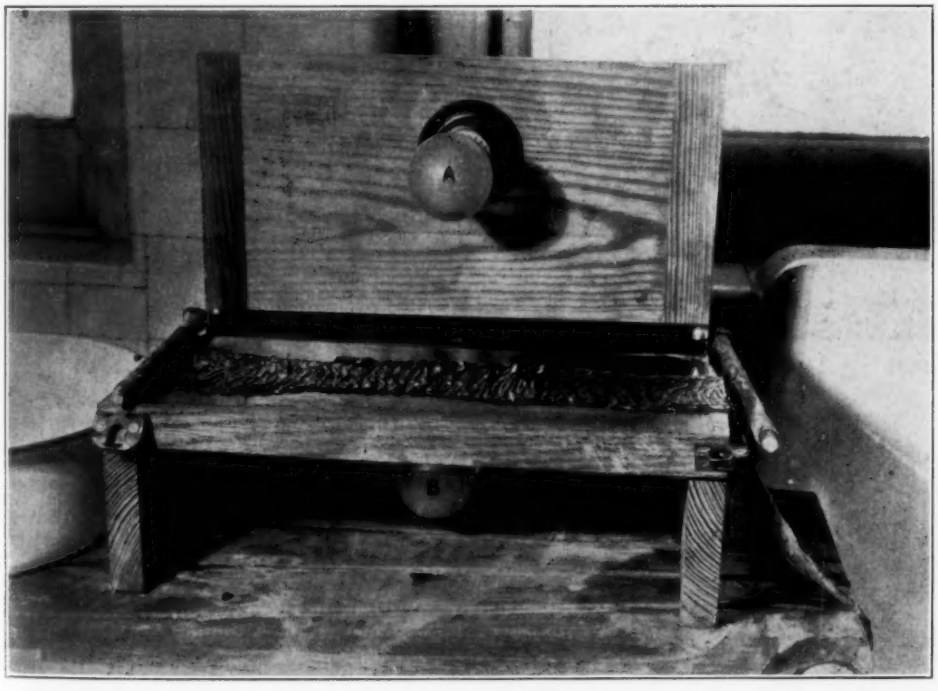

Fig. 1.—Intestinal illuminator.

tion by transmitted light is obtained by means of a 60 or 75 watt bulb situated below the glass plate (*B*), thus producing a transparency. The wood is rendered impermeable to moisture by immersion in hot paraffin. Two hinged iron rollers,

*From the Department of Laboratories and Medical Research, Bronx Hospital.
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located on either end of the viewing table (i.e., the glass plate), are covered with rubber tubing and serve to hold the opened intestine flat. In routine use as pictured in Fig. 1, the opened washed intestines are brought to the drain board in a basin which may be seen to the left of the intestinal illuminator. The intestines are then fed to the glass plate with mucosal surface uppermost and viewed by reflected light (upper bulb *A* only is lit), the examined portions of the intes-

Fig. 2.

Fig. 3.

Fig. 2.—Focal hemorrhagic necrosis ileum (arrow) above which a Peyer's patch can be faintly discerned.

Fig. 3.—The same specimen as in Fig. 2 viewed as transparency. Note lesions located at ends of arborizations due to vascular necrosis at these points.

tine passing under the second roller and falling into a basin located in the sink. When examination by reflected light has been completed the specimen is reexamined in the same way by transmitted light (lower bulb *B* only is lit). Measurements of lesions or their location may be made by means of a ruler graduated in centimeters attached to the board at the far edge of the viewing table. We

use a strip of old film graduated as above with a distinguishing mark for every 5 cm. After a little practice, examination by both reflected and transmitted light

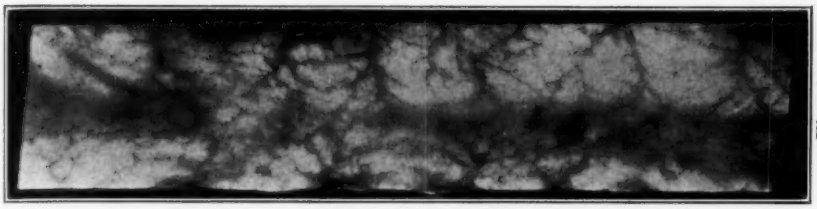

Fig. 6.

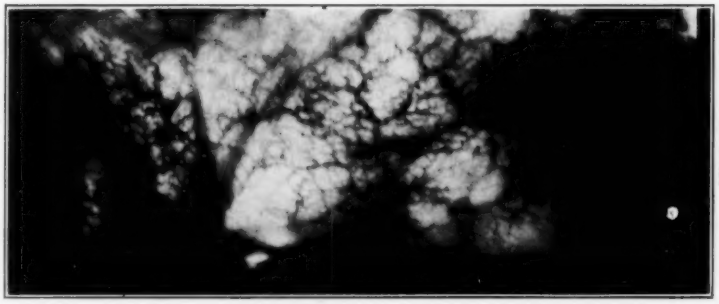

Fig. 5.

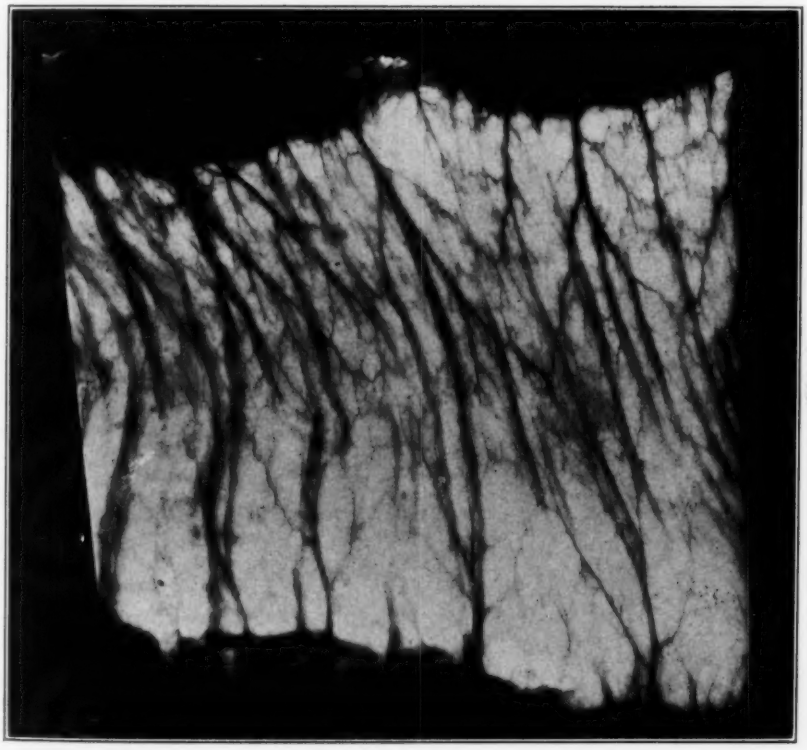

Fig. 4.

Figs. 4, 5, and 6.—Other transparencies.

may be completed in the same operation, the examiner switching off either light as conditions warrant.

This apparatus enables one to detect many intestinal lesions which are missed or not visible in reflected light (see Figs. 2 and 3). A comparative table is given.

Intestinal pictures seen by:

REFLECTED LIGHT	TRANSMITTED LIGHT
Peyer's patches	Blood vessels
Focal lymphoid hyperplasia	Vascular and perivascular lesions
Projecting tumors	Emboli
Ulcers	Focal necroses
Varicosities	Intramural abscesses
Melanosis	Infiltrating tumors
	Small intramural tumors (e.g., leiomyoma)
	Early lesions in Peyer's patches
	Leucemic infiltrations

A PATHOLOGIC LIBRARY OF GROSS SPECIMENS*

NILS P. LARSEN, M.D., HONOLULU, T. H.

DURING the past few years at the Queen's Hospital we have developed a pathologic library which has received considerable comment from visitors as being a plan that should be published. Although there is nothing particularly original in its concept, the use of ordinary laboratory equipment for the development of the pathologic museum may be sufficiently different from the usual to warrant publication.

We have stressed the fact that a general hospital should be the medical center of any community where there is no medical school. With this in mind, for a number of years we collected interesting pathologic specimens in the usual laboratory jar. These are cumbersome and distasteful to handle and do not show the lesion well. They were very difficult to make use of in our weekly clinics, so we began some years ago using the ordinary Petri dish for the mounting of interesting specimens. The method used is as follows:

The specimen is first fixed in Jaure's solution, through which, during the first hour, illuminating gas is passed. It is kept five days in Jaure's solution, then in running water for five hours, and finally in 80 per cent alcohol. From this solution the specimen is cut to fit the Petri dish. The mounting solution is then prepared as follows: In every 1,000 c.c. of distilled water, 75 gm. of gelatin are dissolved. To every 200 c.c. of the gelatin mixture one adds, as the solution is cooling, 25 c.c. of a 40 per cent formalin. As soon as the formalin is added to the gelatin, it must be immediately poured over the specimen. It hardens rapidly. After the specimens are mounted in the above solution and have stood overnight, a thin layer of beeswax and paraffin (1 to 2) is applied. After this is thoroughly dry, a thin layer of plaster of Paris is poured over and levelled. Finally, when this is set and thoroughly

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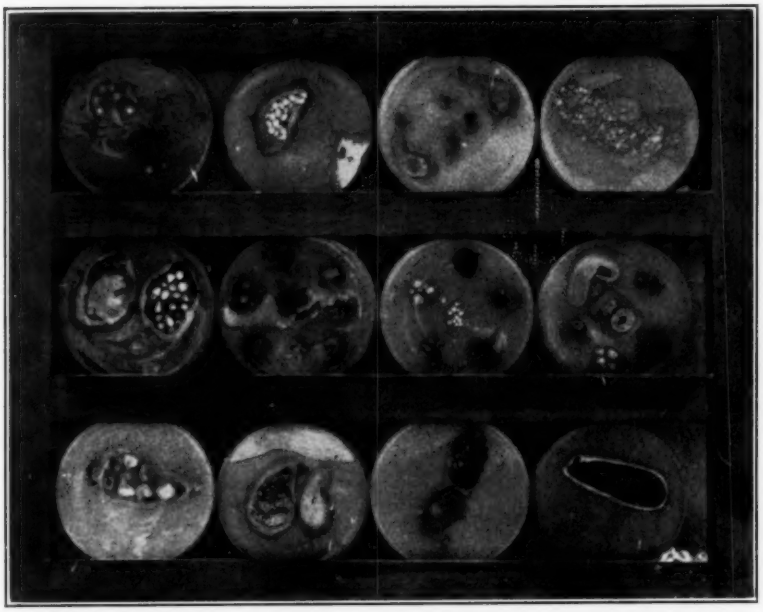

Fig. 1.—Specimen page from pathologic library.

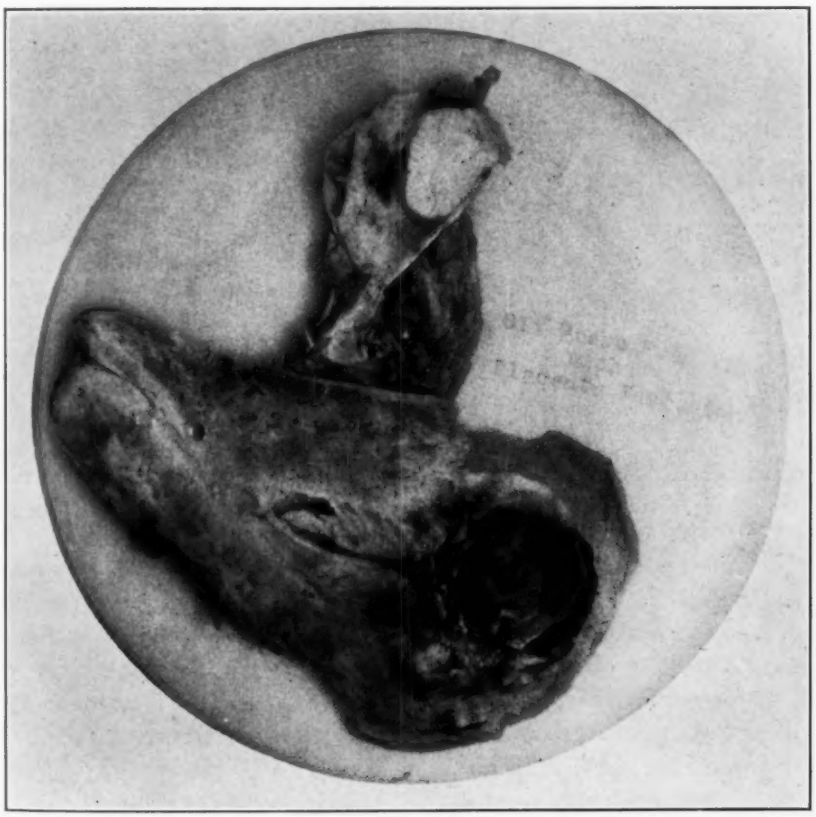

Fig. 2.—Uterus, ovary with corpus luteum, six-week embryo.

dry, another coating of beeswax and paraffin is applied. Recently we have tried battleship cement to seal the plaques. We believe this will make them more permanent. A typewritten caption can be placed in the gelatin as soon as it is hardened.

The house carpenter then built what we call the "pages" for our pathologic library. These are illustrated in Fig. 4. It will be noticed that the plaques can be slid in from the side, so that each "page" contains twelve plaques. Any one can be removed individually and can be handled with impunity. The student or doctor has a more intimate contact with the specimen and can appreciate more fully the gross pathology. Figs. 2 and 3 are of individual plaques. Micro-

Fig. 3.—Cross-section of heart with coronary sclerosis in case of sudden death.

scopic sections are also made and filed with numbers. Hence the microscopist, while he is studying a section, can have a plaque with the gross specimen by the microscope. This, we believe, is of importance, especially to the young pathologist. Fig. 4 shows our whole library as it is arranged in wooden frames above our ice box. It is needless to comment on how much space is saved in such a pathologic museum. A second advantage is, if the pathology or the disease of a certain organ is to be discussed, the whole page showing the variations of pathology in this particular organ can very easily be carried to the clinic room. They are also used in classroom teaching for the nurses.

The total cost of materials and mounting of such a specimen is forty-two cents. If air should get into it, it can very easily be remounted with a minimum of added expense. If air-tight, these plaques have kept very well for several years.

Another use we have made of these is to lend them out to biology teachers of high schools, especially our plaque containing a series of human embryos.

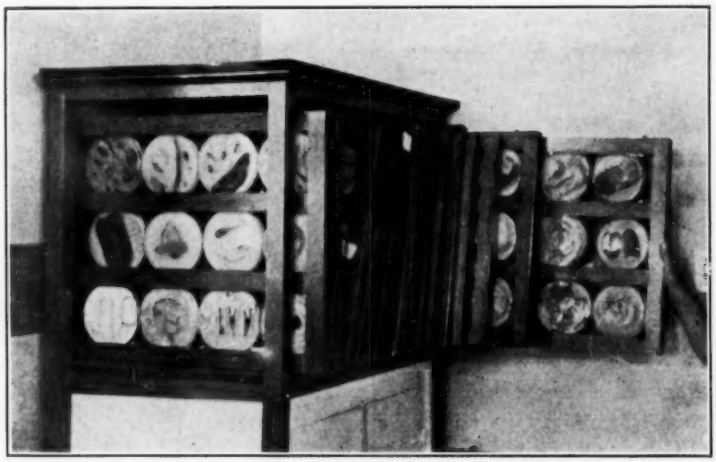

Fig. 4.—Pathologic library complete.

We believe the teaching of biology from human specimens is more interesting than the teaching of biology from bugs and grasshoppers.

A general hospital, if it lives up to its duty as a medical center, should be prepared to help the teachers of the various schools, which can be done by making use of the material available. The hospital laboratory should be prepared to help the schools with all types of laboratory material, and be one of the links in teaching its community what sane, scientific medicine really is.

A RAPID UNIVERSAL BLOOD STAIN*

May-Gruenwald-Giemsa in One Solution

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ONE of the commonest and one of the most important daily performances in a clinical laboratory is staining of blood smears. With growing interest in the prognostic and diagnostic value of the leucocytic picture, the necessity of obtaining uniformly well-stained blood smears is obvious. In addition to this, uniformity in staining smears is the first step toward a much needed standardization of blood cell classification. However, in the average clinical laboratory blood smears are inadequately stained and great difficulty experienced in obtaining uniform results. The causes for this common and serious failure are multiple, and it would be beyond the scope of this article to enumerate them. As a rule it is not the inadequacy of the original material but rather the method of employing it which is at fault.

Most of the methods now employed are based on the Romanovsky method which was first introduced in 1891. Of the very numerous modifications which followed, the best is that of Giemsa, although in this country the methods of Wright and of Wilson are more popular, probably because simpler. Giemsa obtained in pure form ethylene azure which is usually known as Azure I. A mixture of equal parts of Azure I and pure methylene blue is called Azure II. In the Giemsa solution this is mixed with Eosin R.A., heated with glycerin and then dissolved in pure methyl alcohol. Pappenheim proposed fixing and staining of the smears with the May-Gruenwald solution (a methylene blue eosin mixture) and then restaining them with diluted Giemsa solution. This method is by far the best that we have at our disposal, correcting the faults of either method, namely the pale color of the erythrocytes and the pale color of neutrophilic granules with the Giemsa alone and the failure of the May-Gruenwald to stain adequately the azurophilic granulations and the tendency to overstain the nucleus and neutrophilic granulations with resulting loss of important details. Ferrata endorses the same technic with minor changes.[1]

The objections to this method are the high cost of the Giemsa solution, which is rather difficult to prepare in the ordinary laboratory, the necessity of using perfectly neutral distilled water, the rather complex technic that allows staining of only a few slides at a time and finally the time required, which is about twenty minutes. The rapid Giemsa method employing a mixture of Giemsa and methyl alcohol or Giemsa acetone in equal parts is a quick and satisfactory staining method for routine work.[2] Employing a buffered solution for the dilution

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of the Wright stain according to Haden[3] has improved the uniformity of results with this solution, without, of course, removing fundamental deficiencies of this stain. These are similar to those mentioned for the May-Gruenwald, with the added tendency to form precipitates on the smear. The Wright stain has by some workers been substituted for the May-Gruenwald in using the Pappenheim technic.

It appears therefore very desirable to combine, if possible, the staining properties of the Giemsa method with those of the May-Gruenwald in one solution, simplifying the preparation of the stain as well as its use, at the same time standardizing the procedure so as to obtain exactly the same result with each smear. This may be accomplished by using a stain prepared as follows: 1.3 gm. of finely ground Azure II-eosin mixture (Giemsa) are suspended in 80 c.c. of pure glycerin and allowed to dissolve, shaking occasionally, for two or three days. This mixture is then heated in the water-bath at 60° C. for two hours, stirring once in a while. The mixture is then cooled and dissolved in the following: methyl alcohol, 290 c.c., acetone C.P. 290 c.c. Prepare separately the following solution: methylene blue eosin powder (May-Gruenwald) 15 eg., methyl alcohol 170 c.c., acetone C.P. 170 c.c. It takes several days for the powder to dissolve, shaking occasionally. When entirely dissolved add to the Azure II-eosin solution and mix. Occasionally not all of the Azure II-eosin dissolves. It is better to allow it to remain in the bottom of the bottle. It will finally dissolve and if not, it does in no way interfere with the staining property of the solution. The technic for staining is as follows: fresh thin blood smears are covered with the stain and fixed under cover to avoid excessive evaporation for two minutes. Use 1 c.c. of stain. After two minutes add 1 c.c. of the alkalinized solution. Mix *thoroughly* and allow to stand for three minutes. Wash under strong running tap water. Air dry and examine. The alkalinized solution is prepared from a stock solution of 1 per cent sodium carbonate in distilled water. Two-tenths cubic centimeter of this solution is added to 100 c.c. of distilled water. This is used for the dilution of the stain on the slide as indicated.

The 1 per cent stock solution of sodium carbonate should be renewed every two months; the diluted solution should be renewed every two or three days.

The preparation of this stain is simple and allows no variations. Its use is rapid and easy, there is freedom from precipitate, and overstaining is not easily done. The stain keeps well, the only thing requiring some attention is the amount of alkali to be employed. In this regard it must be said that dyes from various manufacturers and even different batches of dyes from the same manufacturer vary slightly.

When the solution is first prepared, it is best to allow it to stand for a fortnight or so before determining the amount of alkali necessary, if any.

If the stain is too acid, the color of the slide is bright red, and on microscopic examination the erythrocytes are found to be very bright red, the eosinophilic granules are likewise overstained but the basophilic cytoplasm of lymphocytes and monocytes is practically colorless, the neutrophilic granules are muddy and indistinct, the azurophilic granules are faintly stained, and the nuclei also are very pale. When the solution is too alkaline the smear appears bluish and

microscopic examination shows purplish blue erythrocytes, opaque purplish eosinophilic granules, large overstained neutrophilic granules and azurophilic granules, nuclei very dark with loss of detail and basophilic cytoplasm of lymphocytes and monocytes too deep blue. When the diluent added is of proper reaction, an iridescent scum appears over the stain. This does not give rise to precipitates and is easily washed off.

In a typical experiment stains were prepared with dyes from five manufacturers. Of these three proved satisfactory, two being domestic and one a foreign product. The three satisfactory stains were allowed to stand for fifteen days and then tested. "A" required for diluent 0.1 c.c. of 1 per cent sodium carbonate solution, diluted with 100 c.c. of distilled water. "B" required nothing but distilled water. "C" (the foreign product) required 0.2 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. Six months later these same stains were tested again. They had been kept in cork-stoppered brown glass bottles. At this time "A" required 0.2 c.c. of the 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "B" required 0.1 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "C" required 0.3 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. It was also found that the time of staining for "C" had increased from three to five minutes.

A year later the dyes were tested again with the result that "A" required 0.6 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water for staining, "B" required 0.3 c.c. of a 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water, and "C" required 0.4 c.c. of a 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "C" required seven minutes with the diluted stain, whereas with the other stains, a three-minute period was sufficient. After about two years from the date of preparation, stain "A" required 1 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water for the diluent. The stain was allowed to remain for five minutes. Stain "B" required 0.6 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water for the diluent, and five minutes for staining. "C" required 1.2 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water as diluent, the staining time being seven minutes. After the two-year period the stains begin to deteriorate and the first sign of a permanent loss of staining qualities is the inability to differentiate properly the neutrophilic granules. It may be stated then that except for variations in the concentration of the solution of sodium carbonate used as diluting fluid, the stain can be safely used for a period of one and one-half years from the date of preparation, after which it must be more carefully watched. Solutions two years old or more are not dependable.

This experiment has been repeated and similar results were found on several occasions, namely that as the solution matures it requires slightly larger amounts of alkali and longer time to obtain the same staining. All of the other staining properties remained absolutely unchanged. Tests carried out with numerous other blood stains show that all of them require with aging a change in the diluent. As a matter of fact, the stain here proposed is more stable than any that

we experimented with including the best commercially prepared solutions of the Giemsa stain. If the stain is prepared in small amounts, sufficient for a period of three to six months, hardly any variation in the amount of alkali is required after the first fifteen days. If variations in the amount of alkali are necessary, they are, as one can see, very easily done. As a matter of fact, the possibility of completely controlling individual features of the stain by varying the amount of alkali, and yet reproducing the same general results, is a very important feature of this method. Great latitude is also allowed in the time of staining, although as a rule three minutes of the diluted stain are sufficient; five and even ten minutes will not overstain. In determining the minimum time and the dilution, we have taken mainly into consideration the staining of the nucleus which we consider the essential feature of most cells. The nucleus, in three minutes, will be found stained perfectly, showing the finest details of the chromatin network, as well as coarser structures such as nucleoli, etc. Monocytic nuclei show particularly good differentiation, which makes the cell readily recognizable.

Practically, the titration of a newly prepared batch of stain is carried out as follows: a number of slides of normal blood are covered with 1 c.c. of the stain. After two minutes the diluent is added, 1 c.c. being used, with the following concentration: Slide 1, 1 c.c. of pure distilled water; Slide 2, 1 c.c. of solution prepared by adding 0.1 c.c. of a 1 per cent sodium carbonate solution to 100 c.c. of distilled water; Slide 3, 1 c.c. of a solution similarly prepared, using 0.2 c.c. of the 1 per cent sodium carbonate solution to 100 c.c. of distilled water and so on to a concentration of 0.5. The slide giving the best staining qualities indicates the best concentration for that particular batch of stain. None of the stains showed precipitate on long standing. When the stain is well applied, the slide is grossly of a bright pink purplish color, and at the microscope shows a pleasant pink purplish color of the red cells. Platelets are fully stained, the cytoplasm of lymphocytes and monocytes is pale, transparent blue, allowing a good differentiation from the deeper blue of the Tuerck cells, plasma cells and immature erythroblasts and megaloblasts. The nucleus of the polys and that of the monocytes is lightly, delicately but fully stained showing the structure very distinctly. That of the lymphocyte, of course, is proportionately much heavier stained. The fine azurophilic granules of the monocytes as well as azurophilic granules of the mature lymphocytes and other cells are fully stained. The neutrophilic granules appear very minute and lightly stained in a purplish pink color, allowing a very easy differentiation between the normal granulations as described and the hyperchromic forms occurring in infection (toxic granulations). The abnormal cells, both the immature and the undifferentiated, as well as degenerated ones, are readily stained within the ordinary time. As a rule, bone marrow smears should be stained for a little shorter period, about two-thirds of that required for ordinary blood smears.

In addition to staining of blood smears, this stain has been found very satisfactory for the staining of malarial parasites, blood parasites in general, and spirochetes (by staining twelve hours in a Coplin jar, with stain diluted 1:4). We would like once more to emphasize the essential requirements for obtaining

good results as being: first, fresh thin smears; second, exactness in the preparation and dilution of the stain; third, correct amount of alkali; fourth, thorough mixing of stain and diluent.

SUMMARY

A method is given for the preparation and use of a rapid universal stain for blood smears, combining in one solution the staining properties of the May-Gruenwald and of the Giemsa solutions.

NOTE: The stain described in this article is now prepared by the Coleman and Bell Co., and submitted to strict standardization before distribution.

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A RAPID FLOCCULATION METHOD FOR THE DIAGNOSIS OF SYPHILIS*

FINAL REPORT

F. RYTZ, MINNEAPOLIS, MINN.

THIS flocculation method, as applied to blood and spinal fluid samples submitted to this laboratory by the committee on evaluation of serodiagnostic tests for syphilis in the United States,[2] has been reported in an earlier publication.[1] A few more or less important modifications are included in this detailed report.

In working out the present rapid flocculation method, mainly two time-consuming factors were to be overcome, the coagulation of the blood and the traditional thirty-minute heating of the serum. It is known that certain proteins, such as peptone in 1 per cent solution, when added to fresh blood, will cause it to coagulate in a few minutes, but when antigen is added to serum derived from such a blood sample, flocculation may take place in normal serum. Heating, however, serves the purpose of coagulation well if the blood sample is placed in a water-bath near, but not above, 60° C., especially if the tube containing the sample is lightly rotated in the bath as motion of the blood facilitates coagulation. The sample is solidly coagulated in from two to three minutes. Clear serum is obtained by centrifugation at high speed for about two minutes. The heating of the blood is no requirement for the test and is only

*From the Clinical Laboratories of the Minneapolis General Hospital.
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applied in cases of emergency. In no instance does heating of the blood compensate for necessary heating of the serum in order to activate properly the antibody for flocculation of the antigen.

Heating of the serum for three minutes only is insufficient if the serum, soon after heating, is not treated with ammonium sulphate. It is known that some serums will react without heating, but the flocculate tends to dissipate in a very short time. By this method the flocculate will remain intact for several weeks at room temperature, and flocculation readily takes place, especially in treated cases, although the antibody content is very low. The quantity of half saturated ammonium sulphate applied is one part to three parts of the serum. The purpose of the ammonium sulphate is to precipitate minor quantities of serum protein and, together with it, specific antibody as both are precipitated by the same means.

When applied to the globulin fraction alone of syphilitic serum, redissolved in 0.9 per cent sodium chloride solution, this method constantly gives negative or very weak positive reactions, most probably depending on the accuracy with which the globulin fraction is precipitated. On redissolved total protein from syphilitic serum, strongly positive results are uniformly obtained. On protein from negative serum, negative reactions result.

THE ANTIGENS

An emulsified antigen[1] of high lipoid concentration appears necessary for a flocculation reaction in serum prepared as for this test. The antigen is prepared as follows: One hundred grams of Difco beef heart powder are suspended in 500 c.c. of absolute ethyl alcohol and shaken for two hours. The suspension is then filtered through a heavy grade of filter paper into a liter flask. This is tightly stoppered, the cork being protected with tin foil, and stored in the ice box for twenty-four hours during which time a heavy precipitate will form. It is then filtered and the clear extract placed in an evaporating dish and evaporated under an electric fan. A soft brownish wax results. The fanning is continued until the alcohol odor has disappeared, whereupon the wax is dissolved in 175 c.c. of anesthesia ether and shaken in a tightly stoppered bottle for five minutes. This is stored in a dark place at room temperature until the next day. The ether extract is again shaken for a few minutes and then transferred to centrifuge tubes for centrifugation at high speed for five minutes. The clear supernatant fluid is poured into the evaporating dish and fanned for a few minutes until the contents start to thicken. Then 400 c.c. of pure acetone are added. A light colored waxy precipitate forms which gradually turns brown. The wax is stirred a few times with an applicator and then left to settle for ten minutes whereupon the acetone is carefully decanted. The residue, in the form of a soft, sticky wax, is fanned until the acetone odor has disappeared. Then the wax is weighed and dissolved in absolute ethyl alcohol in the proportion of 1 gm. of wax to 10 c.c. of alcohol. A brown, glass stoppered bottle is preferred for storing the antigen. The wax is dissolved in the bottle by shaking for a few minutes before it is placed in a water-bath at about 55° C. for fifteen minutes. It is then stored in a dark place until the next day and filtered through a filter paper of dense texture. Any undissolved wax which may adhere to the walls

of the bottle is rinsed out with ether before the antigen is restored to the bottle. It is important that the antigen does not get in touch with wood, cork, or rubber as this seems to affect the product unfavorably.

Antigen thus prepared is stable for at least two months at room temperature if protected against light and air.

THE EMULSION

The amount of antigen to be used for a given quantity of emulsion is determined by titration. The emulsion is prepared as follows: Into a 50 c.c. centrifuge tube is measured 0.8 c.c. of 0.45 per cent sodium chloride solution to which is added 1 c.c. of 1 per cent cholesterol in absolute ethyl alcohol. The cholesterol is added very slowly down the side of the tube and then lightly rotated, not shaken, for about ten seconds. The amount of antigen as determined by previous titration, as a rule about 0.1 c.c., is added very slowly and then shaken vigorously for one minute before the addition of 2.5 c.c. of 0.9 per cent saline. This is again shaken for one minute when the emulsion is ready for use. If fresh salt solutions are employed, the emulsion will remain stable for about a week at a temperature not above 20° C. It is kept at room temperature and reshaken before use.

ANTIGEN TITRATION

Five emulsion tubes are prepared as described above. Before final addition to each tube of 0.9 per cent saline, various amounts of antigen are measured into the different tubes, beginning with 0.05 c.c., increasing the quantity with 0.05 c.c. for each tube so that the last tube contains 0.25 c.c. of the antigen.

Three rows of five tubes are then placed in a suitable rack. The size of the tube is perferably 10 mm. by 75 mm. Into each tube of the first row is measured 0.15 c.c. of a known negative serum, and into all tubes of the second row is measured 0.15 c.c. of a known weakly positive syphilitic serum. Into each tube of the third row is measured 0.15 c.c. of a known strongly positive serum. The tubes are then placed in a water-bath at 60° C. for three minutes. After removal from the bath 0.05 c.c. of half saturated ammonium sulphate is added to each tube. The rack is shaken for a few seconds in order to redissolve the protein precipitated by the ammonium sulphate. To the first tube in each row is added 0.05 c.c. from emulsion Tube 1, and to the second tube in each row is added the same amount of emulsion from emulsion Tube 2 and so on until all tubes are accounted for. Then 1 c.c. of 0.9 per cent saline is added to all tubes and the contents mixed by shaking. The tubes are shaken in a Kahn shaking device for three minutes, or by hand at the rate of about 275 oscillations per minute. Finally 2.0 c.c. of 0.9 per cent saline are added to each of the tubes. One by one the tubes are slowly inverted three times by placing the index finger over the mouth of the tube. It is important not to shake the flocculate when inverting the tubes. The results are then estimated, preferably over the Fisher lamp for reading Kahns or a similar contrivance. The slanted part of the contents of the tube is examined with a hand lens in establishing the titer. The tube with the weakly positive serum which shows the largest clumps of flocculate

indicates the amount of antigen to be used for the given quantity of emulsion, provided the corresponding tube with known negative serum remains negative, and that the tube with the strongly positive serum registers positiveness markedly above the tube with weakly positive serum. All three factors are taken into consideration in establishing the titer of each new batch of antigen.

Fair results may be obtained by using 0.1 c.c. of antigen for the quantity of emulsion given above without titration, but chances are that some weakly positive reactions will be missed, or negative reactions may be unfavorably affected for convenient reading.

THE TEST

This method requires only one tube for each serum and the entire procedure can be carried out in fifteen minutes.

The serum unit, 0.15 c.c., is measured into the respective tubes and the rack placed in a water-bath at 60° C. for three minutes. After the rack has been removed from the bath and the serums slightly cooled, 0.05 c.c. of half saturated ammonium sulphate is added to each tube. The rack is then shaken for a few seconds in order to redissolve the precipitated protein, whereupon 0.05 c.c. of antigen emulsion is added to each tube. This is mixed by shaking. Then 1 c.c. of 0.9 per cent sodium chloride solution is added to each tube. The rack is shaken vigorously for a few seconds before it is placed in the mechanical shaker and shaken for three minutes at the rate of about 275 oscillations per minute as described under antigen titration. Before reading the results 2 c.c. of 0.9 per cent saline are added to all tubes. Each tube is slowly inverted three times.

A negative test consists of unflocculated emulsion particles. The degree of positiveness is estimated according to the clearness of the contents, and the distinctness of the individual clumps of the flocculate.

CONFIRMATORY PROCEDURE

In cases of doubt regarding negative or weekly positive reactions, the tubes are centrifuged at high speed (about 2,000 revolutions per minute) for three to four minutes. The fluid is then poured off by quickly inverting the tubes, and 1 c.c. of 0.9 per cent salt solution is added to each tube. Before reading, the tube is inverted slowly once without shaking. In a negative reaction the emulsion particles scatter readily. A positive test consists of large, irregular flocculation particles. Even a very weakly positive reaction is more distinct than a strongly positive test by the routine method of reading. Under no circumstances can centrifugation be substituted for shaking, but centrifugation is a valuable means by which to force from the routinely completed flocculation reaction the slightest degree of positiveness. This procedure has no bearing on the specificity of the test. It prevents, however, misinterpretation of certain negative reactions.

COMPARATIVE RESULTS ON BLOOD SAMPLES

The relative value of this test in comparison with other flocculation and complement fixation methods, as used in this country, has been published by the committee on evaluation of serodiagnostic tests for syphilis in the United States.[2]

In performing this test on samples submitted by the committee on evaluation, an effort was made not to report results as "doubtful." The confirmatory method described above is a recent improvement not applied on evaluation samples. In certain groups, "positive" results based on weak 1-plus manifestations would have been clearly negative with the confirmatory method of reading.

In our laboratory, results obtained with this test have been compared with the Kahn and the Wassermann on 5,322 blood samples, of which 350 were from patients undergoing syphilitic treatment. The method here described proved to be 3 per cent more sensitive than the Wassermann, 1 per cent more sensitive, and also 1 per cent more specific, than the Kahn, as performed in this laboratory.

Results based on the 350 treated syphilitics alone, indicated this test to be 11.4 per cent more sensitive than the Kahn, and about 25 per cent more sensitive than the Wassermann reaction.

SPINAL FLUID

The present method as first applied to spinal fluid proved unsatisfactory on a comparatively large number of samples. Research work now attempted on spinal fluid in this laboratory appears at this time promising for a rapid, sensitive flocculation test for syphilis on spinal fluid, the result of which will soon be published.

COMMENT

Physical examination of the flocculate resulting from this reaction does not indicate that the flocculate from a positive test has a greater mass than the antigen emulsion alone from a negative reaction, provided both are properly removed. By microscopic comparison the emulsion appears more bulky than the combined antigen-antibody as though the antibody causes changes in the lipoid crystals rather than adds to the total mass.

The cholesterol particles of the emulsion appear as semitransparent, angular crystals with distinct outlines and a slight tendency to clumping. The antigen of the emulsion is visible, in part, in the form of brownish globules of various size, some of which are probably too small for microscopic recognition among other emulsion particles. After flocculation by action of the antibody, the particles lose their distinct outlines as if the individual particle had shrunk in size. The clumping appears definite.

Various investigators, among them Schmidt and Tuljtschinkaja,[3] find that antibodies precipitate mainly with the euglobulin fraction of immune serum. O'Meara,[4] however, terms his streptococcus antitoxin flocculate "not a precipitate of protein," but the substance is "a specific streptococcal product." Pacchioni,[5] through his investigation, concludes that bacteria in the animal body are acted upon by enzymes and that specific antibodies are formed in the process of such bacterial destruction. Witebsky[6] reported success in separating syphilitic antibody from its antigen combination. He also found it possible to reunite the antibody with the antigen after separation. Agglutinins have likewise been separated from the protein of antityphoid serum.[7] It has been shown[8] that rab-

bits inoculated with flocculate from human syphilitic serum develop positive flocculation tests and positive complement fixation tests for syphilis, and that control animals injected with antigen emulsion alone remain negative.

In this connection it is of interest that toxin has been flocculated by antitoxin.[9] Anda and Komiyama[10] regard the ability of the antibody to flocculate an antigen as an indication of the potency of the respective immune serums.

Recent investigations, therefore, suggest that serodiagnostic reactions are true antigen-antibody combinations.

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A RING TYPE STERILIZER FOR USE WITH TISSUE CULTURE FLASKS*

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THE usual method of sterilizing the necks of tissue culture flasks is to heat the neck of the flask with a gas flame. Unless very carefully done this procedure causes some breakage of expensive flasks. We have also suffered loss of valuable cultures by contamination after opening flasks for feeding when using this method. The greatest difficulty has been encountered, however, when it becomes necessary to remove the clot for subculture. This is due to the fact that after the clot has been detached from the bottom of the flask for removal it practically fills the neck of the flask as it is being poured out. Unless the neck is sterile inside and on the edge, contamination is inevitable.

It seemed desirable to have a method which would heat the glass evenly on all sides thereby reducing breakage and also control the degree of heating more accurately.

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It was found that these objectives could be attained by inserting the neck of the flask into the end of heavy pipe heated to a dull red by a gas flame.

A 12-inch pipe, capped at the upper end, is inclined at such an angle that the floor of the flask tilts slightly downward on the side away from the apparatus when the neck is inserted. For flasks of ordinary size, the pipe should have an internal diameter of one inch. The flame is adjusted to heat the pipe equally above and below.

Small squares of wet filter paper placed 2 or 3 mm. inside the neck of the flask are charred black in thirteen or fifteen seconds.

The time required to sterilize the flask neck when heavily contaminated was determined by inoculating the outside and the inside of the neck to a depth of 3 mm. with saliva. The neck was then heated for various periods up to fifteen seconds and, after cooling, the contents of the flask were poured into a sterile Petri dish and then transferred to a sterile flask. After incubation the flasks were examined for the presence of organisms. It was found that heating for ten seconds is adequate to sterilize such heavily contaminated necks.

Corks must be removed since they ignite instantly at this temperature. We routinely remove and discard the cork, and after heating, replace it with a sterile one. The cork is placed with a light, twisting motion as soon as the neck of the flask is removed from the tube.

Accidents of the type referred to above have been largely eliminated by this method.

A TECHNIC FOR OBTAINING HUMAN BLOOD FOR TISSUE CULTURE EXPERIMENTS*

JOSEPH T. KING, M.D., MINNEAPOLIS, MINN.

IN 1930 I described the technic used in this laboratory for tissue culture work.† In the technic described for obtaining blood, serum, or plasma, the blood is drawn by arterial cannulation, by a closed system, into 50 c.c. centrifuge tubes with narrow necks. When plasma is desired, heparin solution in suitable amounts is placed in the flasks before sterilization. When collecting serum, small pieces of chemically clean Pyrex glass are placed in the flask instead of heparin. With this technic contamination practically never occurs.

Frequently, however, we have had contaminations when working with serum or plasma brought to the laboratory from the hospitals. It is evident that the prevailing conditions are such that ordinary methods are not as reliable in meeting the extreme requirements as might be desired. It was therefore considered advisable to attempt to modify and standardize the technic mentioned above to make it suitable for venipuncture methods.

*From the Department of Physiology, University of Minnesota.
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†King, Joseph T.: Arch. f. Zellforsch. 9: 341, 1930.

The centrifuge tubes are prepared with heparin or glass fragments and corked. An 18 gauge hypodermic needle is driven through the cork. The needle should be 1½ inches long. The hub of the needle is left ½ inch above the top of the cork. The bore is explored with a wire to be certain it is free of cork fragments. Cotton is wrapped around the neck of the flask, the cork, and the exposed part of the shaft of the needle. The cotton is tied tightly with thread. The top part of the flask is now wrapped with a paper towel in such a way that the paper can be removed by slipping it off without unwrapping. The tubes are sterilized by autoclaving.

Syringes are prepared in glass tubes with needles attached and sterilized by dry heat.

After sterilizing the skin with iodine, blood is drawn into the dry syringe. An assistant removes the paper towel from the tube and presents the exposed hub of the needle to the operator who, after removing the needle attached to the syringe, attaches the syringe to the needle projecting through the cork. The flask is so held by the assistant that the hub of the needle is between the tip of the index finger and the thumb. In this manner it is held with firmness sufficient to permit the operator to attach the syringe with a light twisting motion.

The unit is now held in the vertical position and the blood expelled into the flask. This is done by alternately depressing and raising the plunger. In this way air is removed from the flask as the blood enters.

The needle, still attached to the syringe, is now removed from the cork. The cotton is left in place.

If the flask contains heparin, mixing is accomplished by gentle agitation, avoiding throwing the blood into the top of the apparatus. If it contains glass fragments, the blood is allowed to clot. The flasks are then delivered to the laboratory and centrifuged without being opened. (In the case of collecting serum, the clot is broken up by striking the bottom of the flask against the hand before centrifuging.)

From this point the technic is as described in the above mentioned publication.

The technic seems to meet the existing requirements very well. We have had very little trouble with contamination since instituting this method.

A RAPID FLUSHING-WASHING APPARATUS FOR LABORATORY GLASSWARE*

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A DEVICE used in this laboratory has proved to be so convenient and time saving and also has elicited so much favorable comment from visitors that we decided to publish a description of it in the hope that it would help other laboratories in their dishwashing problems.

The drawing is self-explanatory. The device itself is drawn in solid lines, while the faucet to which it is attached and an A.P.H.A. type dilution bottle, in position to be washed, are shown by broken lines.

The device, as purchased at a hardware store or a beverage supply store for a few cents and called by them a bottle washer,† does not usually have a "press-

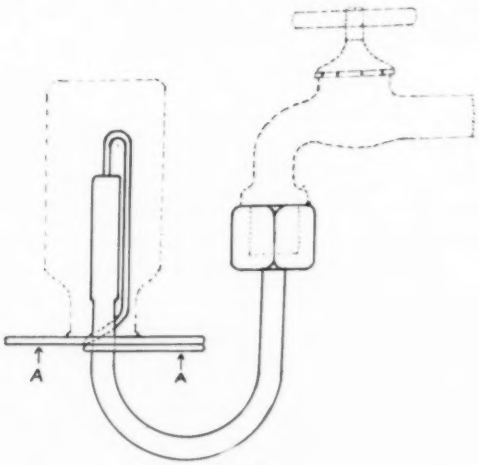

Fig. 1.

ing bar" (*A*) extending all of the way across, hence we soldered one on and covered it with small rubber tubing to eliminate breakage of glassware.

The water pressure keeps the valve shut when the device is not being used, therefore water is used only for the washing or flushing of glassware, and there is no wastage of water.

We use this apparatus for washing or flushing all test tubes (except serum tubes), bottles, flasks, etc. When dirty pieces of glassware first come to the sink, the dishwasher merely inverts them over the device (see drawing), presses them down and holds them there until the material in them is washed out by the force of the water spray. (Pieces of glassware with narrow necks are washed out faster by removing them from the washer momentarily to allow more rapid drainage.) It is surprising how quickly, conveniently, and thoroughly dirty glassware thus can be washed out.

After the glassware has been thoroughly cleaned by use of brush and soap, the soapy water is then flushed or rinsed out from the glassware's interior, quickly and conveniently, by again inverting it over this device and pressing.

*From the Bacteriological Laboratory, Mead Johnson & Co.
Received for publication, August 20, 1935.
†Freedman Bottle Washer.

PROLONGED PRESERVATION OF THE ANTIGENIC SPECIFICITY OF SHEEP CORPUSCLES ASSOCIATED WITH THE PRODUCTION OF A MARKED INCREASE IN RESISTANCE TO HYPOTONIC SOLUTIONS*

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THE tendency of cells to deteriorate is, perhaps, the greatest inconvenience to a laboratory running a complement fixation test for syphilis. Satisfactory preservation of sheep cells for any of the modifications of the Wassermann test is, I believe, generally regarded as a matter of three weeks or less. Kolmer,[1] in the last edition of Kolmer and Boerner, *Laboratory Technic*, claims that cells are satisfactory with formalin for three weeks. With technics in which the strength of the reaction is judged by the amount of inhibition of hemolysis, rather than by the dilution of serum in which complement fixation takes place, as in the case of the Kolmer-Wassermann technic, corpuscles kept for this length of time might not be considered satisfactory. On the other hand, for human corpuscles we have the technic developed by Rous, by means of which blood for transfusion was kept for as long as a month with the addition of isotonic sodium citrate and dextrose solutions. In connection with this technic, Sogen[2] reported an increase in resistance to hypotonic salt solution.

In 1924, Ashby published data showing that corpuscles in solutions in which they lost their normal cation content; for instance,[3-5] human corpuscles which lost potassium, and dog corpuscles which lost sodium, greatly increased in their resistance to hemolysis by hypotonic solutions. This loss of cation content occurred upon prolonged incubation at 36° C. of whole blood in great excess of various isotonic solutions which did not contain the cation normal to the corpuscles. It was shown that the phenomenon of increased resistance to hypotonicity was reversible, if the corpuscles were transferred to isotonic solutions containing the cation normally present. Exceptions, however, were found in certain instances, notably, when an isotonic solution of calcium or barium salts, and, also, if sodium and potassium citrate, had been used as the extracting fluid. It was shown that[3] the increase in resistance to hemolysis was not due to reduction of intracellular osmotic pressure, as the increased resistance still maintained in instances in which it was found by quantitative methods that some other cation had replaced that normally present in equivalent amounts. It was further shown that, in the case of corpuscles whose natural cation content was potassium, the closely related element, rubidium, could be substituted for potassium with a return of a normal fragility of the corpuscle; while, in the case of the dog and cat corpuscles, which normally have a high sodium content, lithium could be substituted for the sodium with the production of normal fragility. It was

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further shown that this phenomenon of increased resistance with the loss of the natural cation could be produced by incubation in appropriate solutions which were above, as well as below, isotonicity.

For these reasons the theory was advanced that the normal cation of the corpuscle bore a chemical relationship, rather than a purely physical one, to the corpuscle hemoglobin stroma complex, and, that the change in resistance to the hypotonic salt solution occurring with the loss of cation was not due to an osmotic pressure effect, but was due to a change in the substance of the corpuscle, resulting in a change in its physical property of solubility in hypotonic salt solution. The probability of the verity of this explanation of the phenomena observed is, I consider, increased by studies, such as those of Van Slyke, Wu, and McLean,[6] indicating that a considerable portion of the base of corpuscles is present as the cation of a salt of protein.

To produce these results, a period of incubation was necessary which approached that causing deterioration of the corpuscles. For instance, in human corpuscles a three-day incubation period in isotonic sodium chloride solution was necessary to produce the loss of potassium giving the maximum increase in the resistance to hypotonic salt solution; while, with four days' incubation, there was evidence of general deterioration of the corpuscle with hemolysis in the isotonic medium. It was found that, although these results could be obtained with defibrinated blood, they were more easily demonstrated with citrated blood. Citrated blood was used in the major part of this work, and the possible effect of the citrate, per se, was adequately controlled.

Brooks[7] attempted to repeat this work of Ashby with the blood of some of the animals used by Ashby, but was unsuccessful in his attempt. For a reason which does not seem to me to be adequate, he avoided the use of citration of the blood to be treated, although the effect is more markedly produced with citrated blood, and substituted defibrination. He also omitted to adjust his extraction solutions to pH 7.4 or 7.6, but used them at pH 6.8. His corpuscles deteriorated with hemolysis into the supernatant fluid within two days, and did not show the effect of increased resistance to hypotonic salt solution. The basis of the discrepancy between the results obtained by Brooks and Ashby, respectively, is not, as yet, clear. In addition to the use of the animal corpuscles reported in the work of Ashby, Brooks used sheep corpuscles without any increase in resistance to incubation in isotonic potassium chloride, as had been found in the case of dog corpuscles by Ashby. I have repeated the attempt of Brooks to produce the increased resistance phenomenon by incubation of sheep corpuscles in isotonic solutions of potassium chloride, and have, so far, been equally unsuccessful. But I find that a marked increase in resistance is produced when sheep corpuscles are incubated in an excess of certain isotonic solutions from which both sodium and potassium are absent; incubation in isotonic sucrose, dextrose, or magnesium chloride produces this effect. I conclude, therefore, tentatively, that the substance of the sheep corpuscle is capable of combining with either sodium or potassium to produce the normal state of fragility, and that the preponderance of sodium normally found in the sheep corpuscle is due to the sodium content of the sheep blood serum.

It was reported by Ashby that human corpuscles, which showed very marked increase in resistance to hypotonic salt solution after incubation in isotonic sodium chloride, were still completely sensitive to antihuman hemolysin in the Noguchi modification of the Wassermann test. This marked increase in resistance to hypotonic salt solution upon loss of the natural cation, which would appear to be produced by decrease in the solubility of the hemoglobin, or, perhaps, the hemoglobin stroma complex, suggested to me a state of dormancy of the cell. It seemed probable that, at reduced temperature at which the lipoid content of the cell would not be so likely to be lost, it might be possible to keep blood cells for a long time. And, since with human cells, at least, it had been found that their antigenic specificity was not lost with this change in their physical properties, the attempt to preserve sheep cells for the complement fixation test was undertaken.

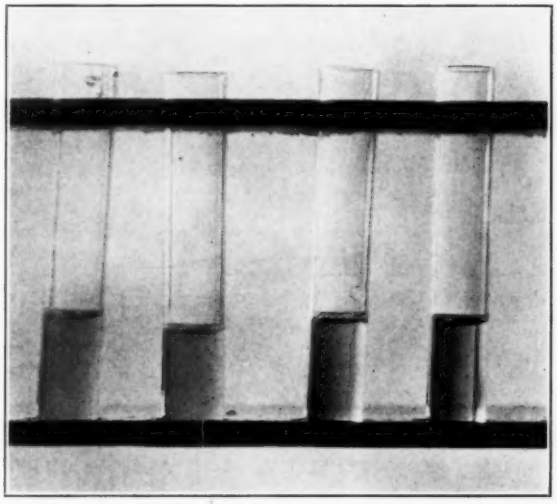

Fig. 1.—Photograph of positive Kolmer-Wassermann test and control run with sheep cells one year and ten days old, illustrating the sharp results obtained with these cells.

Technic and Results.—Attempts were made to preserve sheep cells in hypotonic solutions of sucrose, sodium citrate, dextrose, magnesium chloride, sodium chloride, and mixtures of these with and without the preservatives, formalin and phenol, at room temperatures and at reduced temperatures. Blood was added to the solutions in the proportion of 1 to 10. No adjustment of pH was made. The corpuscles were allowed to sediment and were not resuspended.

The effect of prolonged incubation with formalin was to destroy the antigenic specificity of sheep cells, and, with phenol in preservative amounts, hemolysis took place. With at least certain bacterial growths, specificity was lost, although, in certain instances where a superficial growth of molds had taken place, sensitiveness to hemolysis by antisheep hemolysin was still normal. In view of the fact that preservatives were unsatisfactory, it was necessary to resort to a rigidly sterile technic in taking the blood. The blood was aspirated into a sterile flask to which a bleeding needle was attached with a rubber tubing,

which had been coated with paraffin oil. The whole set-up was autoclaved before use, and the neck of the animal was shaved and sterilized before operation.

It was found that preservation at room temperature was not successful, as hemolysis took place after several days. The use of a domestic refrigerator of a type that underwent considerable vibration when the motor was running and was subjected to a periodic rise in temperature, incident to defrosting, was not so satisfactory as the laboratory refrigerator, which is an adjunct to the morgue, free from vibration, and, since it is not under independent thermostatic control, receives an auxiliary supply of ice and is constantly at a temperature of approximately 3° C. The domestic refrigerator, however, gave useful results.

It was found that cold storage in any one of the above mentioned isotonic solutions, except sodium citrate, resulted in a considerable amount of hemolysis into the supernatant fluid, especially immediately above the corpuscles. With mixtures of isotonic sucrose and citrate, dextrose and citrate, and magnesium chloride and citrate in the proportion of 9 to 1, this deep staining of the supernatant fluid immediately above the corpuscles did not occur, but only a very gradual and general increase in color from straw to pale pink, even after many weeks of storage. The cells in all instances acquired a brilliant brick red color. In citrate, alone, the same results obtained, as far as freedom from hemolysis into the supernatant fluid is concerned, as in the mixtures of sucrose and citrate, dextrose and citrate, and magnesium chloride and citrate. Cells which had been in cold storage in a mixture of isotonic sucrose and citrate, 9 to 1, for more than twelve months did not show an intense reddening of the supernatant fluid.

The increase in resistance which accompanies cold storage incubation in these solutions is marked. The following results were obtained with sheep's blood incubated for six months in a sucrose citrate mixture (Table I). Similar results were obtained with corpuscles incubated in isotonic dextrose, magnesium chloride, and sodium citrate solutions.

The specificity of the corpuscles was tested with antisheep hemolysin in the complement titration and in the complete test, as used in the Kolmer-Wassermann technic. Cells incubated in the sucrose citrate mixture, 9 to 1, were tested after various intervals, varying from six weeks to three hundred and

TABLE I

INCREASE IN RESISTANCE TO HYPOTONIC SALT SOLUTION. CELLS INCUBATED FOR SIX MONTHS IN ISOTONIC SUCROSE SOLUTION AT 3° TO 8° C.

Percentage salt solution	0.085	0.17	0.255	0.34	0.425	0.510	0.595
Fresh sheep cells					4	1	0
In sucrose six months	4	2	1	1	Trace	0	0

seventy-five days. In some instances the cells were merely centrifuged to pack for measurement of volume, and, after removal of the supernatant fluid, they were diluted with 0.85 per cent saline to the standard cell suspension. In other instances they were washed in the usual manner. As compared with fresh corpuscles, there is more initial hemolysis found in washing these cells, but the final result is a clean residue of corpuscles which, when diluted, compare very

favorably with a fresh corpuscle suspension. The chief difference seen between these and the fresh corpuscles is a tendency to sediment rather more rapidly. Washed cells were tested after incubation in dextrose for one hundred and sixty-five days, in sodium citrate for eighty-one days, and in magnesium chloride for fifty days. The results, both of the complement titration and the Kolmer-Wassermann test, paralleled the results with fresh cells, giving practically the same readings; the negatives and controls were cleanly hemolyzed, and the tubes in which there was complement fixation showed turbidity equal to that produced with fresh corpuscles. Below are given readings of the Kolmer-Wassermann test on a series of spinal fluids run with fresh blood corpuscles and with washed corpuscles one year and ten days old (Table II). The results are practically identical, except in one instance, Case 6, which was a treated case of paresis. It showed a weak reaction with the year-old corpuscles, as compared with a negative result with fresh corpuscles. The photograph below shows a three-tube test of a positive blood with the control tube, run with the one-year-old corpuscles, which also gave results identical with those obtained using fresh corpuscles. Smears of the one-year-old cells showed normal staining with Wright's stain. About 50 per cent were normal as to shape; the rest had the form of flattened hexagons and blunt needles, somewhat resembling the cells seen in sickle-cell anemia.

TABLE II

THE CONCURRENCE OF THE KOLMER-WASSERMANN TEST RUN WITH FRESH SHEEP CELLS AND CELLS KEPT ONE YEAR IN ISOTONIC SUCROSE

SPINAL FLUID	KOLMER-WASSERMANN TEST WITH	
	FRESH CELLS	CELLS ONE YEAR OLD
1	00000	00000
2	44200	44200
3	00000	00000
4	00000	00000
5	00000	00000
6	00000	41000
7	00000	00000
8	00000	00000
9	44000	44300

Discussion.—The effect of the addition of citrate to the sucrose, dextrose, and magnesium solutions was to prevent the intense coloring of the supernatant fluid immediately above the settled corpuscles. When the citrate was not used, however, the mass of the corpuscles remained in good condition for long periods. As to how the citrate functioned, was not determined. It is quite possible that the citrate merely maintained an optimum pH, which, in the case of the specimens to which citrate was not added, was produced by hemolysis of the upper layers of corpuscles. Quantitative sodium determinations were not done upon the preserved corpuscles to see whether there was a loss of sodium, but loss of sodium, as an explanation of the increased resistance to hypotonic salt solution, is not ruled out by the fact that resistance increased upon incubation in sodium citrate. Because of its size and structure, the citrate ion might be unable to enter the sheep cells, in which case it would tend to hold back the sodium ion, so that the net result would be the same as that brought about by solutions containing no sodium.

Summary.—A method has been developed for the prolonged preservation of the antigenic specificity of sheep corpuscles.

Results by the Kolmer-Wassermann test comparable to those with fresh cells have been obtained with sheep cells more than a year old.

Prolonged preservation has been accomplished by diluting sheep blood, 1 to 10, with isotonic solutions of sucrose, dextrose, magnesium chloride, and sodium citrate and maintaining the dilutions at 3 to 8° C. Whole blood kept as a control shows signs of deterioration in four weeks.

This development of resistance to autolysis is associated with a great increase in resistance to hypotonic salt solution, and, on the basis of previous work with corpuscles at body temperature, the opinion is expressed that the two phenomena are probably associated with loss of sodium combined with the cell substance.

I wish to acknowledge the technical assistance of Miss Anna Rafferty and of Miss Emera Johnson.

NOTE: Based on work done since this paper was submitted for publication the following suggestions are made for use of the preserved cells in a complement fixation test: The blood should be stored in 3.8 per cent sodium citrate solution. The day before it is to be used the isotonic sodium citrate should be replaced by 0.5 per cent sodium chloride solution. The corpuscles should be resuspended and the mixture returned to the ice box. It will be found that corpuscles so treated wash readily and are free from the undesirable qualities mentioned in the text. With the use of this technic it is unnecessary to bleed the sheep more than four or five times a year. A more complete report will be made on this point.

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A CONVENIENT AND RELIABLE INSTRUMENT FOR ELECTRICAL STIMULATION EXPERIMENTS*

JOHN A. MYERS, M.D., BALTIMORE, MD.

IN AN attempt made in this laboratory to delimit the motor cortex by electrical stimulation, it became apparent that factors producing great variability in the results of such stimulations existed. This variability in results was found not only in different species, but also in the same species and in the same animal during a single experiment. Even after the factors of temperature and of depth of anesthesia were controlled, some factor of variability remained. This factor could then be ascribed either to the uncontrollable variation in the state of the living tissue itself or to the lack of adequate quantitative measurement of the electric current furnished by the type of instrument used to elicit movement.

To ascertain whether these variations were irradicable because they were inherent in the central nervous system or whether the type of stimulating instrument itself contributed an appreciable source of error, the characteristics of the inductorium were studied. The inductorium has been used as a source of energy for the greater proportion of all stimulations of the cerebral cortex. This instrument has a nonuniform calibration scale with very coarse adjustment; its frequency-setting and stability are poor with cessations of operation due to burning and sticking contacts; the regulation of the instrument is unreliable, for it makes the stimulating voltage a function of the load-resistance rather than of the scale-setting; the wave-shape approaches a triangular form but is inconstant and varies with the magnetic saturation of the iron core and with the vibrator frequency. As these disadvantages are characteristic of this type of instrument a new one was designed and tested upon all of the common laboratory animals.

This instrument was a simple potentiometer circuit designed to be operated directly from a 60-cycle, 115-volt lighting circuit. The wave-form of the voltage is practically a sine function, so that the maximum value is $\sqrt{2}$ times the effective value (the effective value being that value read by all commercial alternating current meters). There are no contacts to burn or stick, no disturbing noise, and the frequency is constant for indefinite periods of time. Since the stimulating voltage is derived from the voltage-drop along a resistance wire, the calibration is a uniform scale and may be made by ordinary linear measurements. Once the total voltage-drop of the wire is known, this scale can be read directly in volts and subdivided as many times as desired. The current flowing through the wire is adjusted so that its value is very large compared to the stimulating

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current, and therefore the regulation of the instrument is excellent. Thus the voltage measured on open circuit is the same as that at any particular instant during stimulation.

This instrument may be constructed in any one of three forms, all of which are in use in this laboratory. The simplest form consists of a resistance-wire of uniform diameter stretched between supports placed at each end of a board. An ammeter and several resistors are placed in series with the wire so that the instrument may be plugged into an outlet of a 115-volt lighting circuit. A hot-wire type of current meter is employed, as by its use the voltage-drop of the

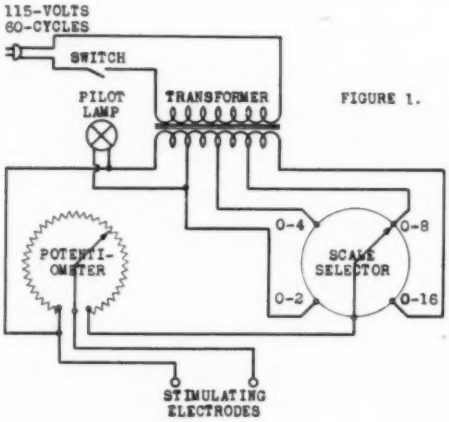

FIGURE 1.

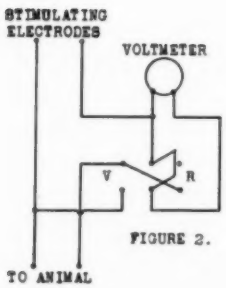

FIGURE 2.

wire may be measured on direct current with a d.c. voltmeter, and the same scale reading of the ammeter on alternating current will give the corresponding *effective* values of the a.c. voltage-drop along the wire.

The second form of this instrument is made on the same principle, the difference being that the straight wire is replaced by two variable rheostats, one 7 ohms, the other 50 ohms, giving fine and coarse adjustment, and the voltage output is measured directly by a sensitive voltmeter. This form requires less space but has the disadvantage that its fineness of calibration is limited by that of the meter, whereas the calibration of the open wire may be spread out indefinitely with slight changes in construction. This form possesses one ad-

vantage not possible with the former in that the resistance of the part under stimulation may be measured quickly at any point desired and the value of the stimulating current calculated.

The third form is that which is most suitable for general use both for experimental investigation and for clinical work. A functional schematic diagram of the instrument is shown in Fig. 1. The circuit consists of a transformer with a tapped secondary winding which supplies 2, 4, 8, and 16 volts to respective taps on the rotary switch designated "Scale Selector." The voltage of the tap selected is connected across the potentiometer. This fixed voltage impressed across the potentiometer resistance is then varied continuously by movement of the potentiometer contact arm through an arc of 315 degrees. The variable arm of the potentiometer is connected to the output terminals marked "Stimulating Electrodes," so that 0-2, 0-4, 0-8, or 0-16 volts may be obtained for stimulation. Four complete calibration scales are engraved on the panel to designate the intermediate voltage settings of the potentiometer. The full-scale values are correct with a line voltage of 115. This gives sufficient accuracy for most work; however, if there is considerable variation in the line voltage of the lighting circuit it is a simple matter to check its value and apply a correction to the calibration scale.

If it is desired to measure the resistance of the part between the stimulating electrodes a Weston Mod. 301 Universal A.C.-D.C. meter may be connected into the circuit as shown in Fig. 2. With the switch in position marked *R*, the resistance is determined; in position marked *V*, the voltage at the electrodes is read by the meter. Thus it is possible to obtain quickly and easily the stimulating current by applying Ohm's law $I = E/R$. Measurements of this kind have shown that the resistance of an animal, measured between the pia mater and the skin over the lumbar region, is approximately 2,500 ohms and remains remarkably constant for as much as four hours of continuous stimulation. This resistance does vary according to the type of animal and also between different members of the same species. However, it has been found that the strength of the stimulating current for a species is quite constant regardless of resistance variation. The strength of current required to elicit movement is, however, greatly affected by depth of anesthesia. These points will be discussed more fully in another paper.

SUMMARY

A simple alternating current potentiometer is described; this instrument deriving its current from a 115-volt, 60-cycle lighting outlet overcomes all the disadvantages of the inductorium.

A STUDY OF THE RELATIVE SENSITIVITY AND SPECIFICITY OF THE KOLMER-WASSERMANN, KAHN AND EAGLE PRECIPITATION TESTS*

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THE present study was undertaken to compare the Kolmer-Wassermann test, the Kahn precipitation test, and the Eagle precipitation test with respect to sensitivity and specificity, using carefully studied known cases of syphilis for the former, and a normal nonsyphilitic population for the latter. All three technics were carried out as routine tests on the serums used in our study, after first familiarizing ourselves with the Eagle test, which has been but recently added to our routine serologic procedures.

The results obtained by the serologists who participated in the serologic conference recently conducted by the U. S. Public Health Service and the American Society of Clinical Pathologists represent results under comparatively ideal conditions. The tests were carried out by the serologist who devised them and do not necessarily reflect the experience of the average routine diagnostic or hospital laboratory. Our results are therefore described as representative of what may be expected of some of these tests in the hands of the average skilled technician.

MATERIAL AND METHODS

Patients under study or treatment in the syphilis clinic, or syphilitic patients originating in other services comprised the clinical material used as a criterion for sensitivity. Studies for specificity were conducted on a series of selected normal individuals, considered nonsyphilitic on the basis of past history and social status.

Serologic Tests.—The Kolmer-Wassermann test was performed with 0.2 c.c. of serum as the maximum amount as suggested by Boerner and Lukens,[1] the second and third tubes containing 0.1 and 0.05 c.c., respectively. The antigen was the one recommended by Kolmer at the time of this study, and the test as performed has had Kolmer's approval.[2] The Kahn precipitation test was conducted as described by Kahn,[3] with antigen furnished by Dr. Kahn and also with antigen prepared in this laboratory. Results showed both antigens to be equal in sensitivity and specificity. The Eagle test was performed according to Eagle's[4] technic with antigen furnished by him. As this test has been used by us but a comparatively short time, we ran approximately 1,000 tests before starting the study of comparative tests. The earlier readings were in many instances read or checked by Dr Eagle.

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Positive readings were given the Kolmer-Wassermann of plus-one (+1) or stronger. Doubtful readings were those of plus-minus (±). Kahn readings of average plus-one (+1) or stronger were considered positive, while readings of average plus-two-thirds (+⅔) were classed as doubtful and those of plus-one-third (+⅓) as negative. A positive Eagle reading was that in which definite coherent aggregates were formed, persisting on the addition of salt solution; doubtful readings were those in which there was questionable aggregation of the crystals even after recentrifugation as recommended by Eagle.

CLINICAL AND SEROLOGIC RESULTS

A total of 2,730 tests in triplicate were done on as many patients subsequently grouped as follows:

Group A.—All tests negative. This group was comprised of proved nonsyphilitic cases, of cases of infection too recently acquired to have demonstrable antibodies by any of the three tests, and of syphilitic cases which had become serologically negative under treatment. This group gives merely the number of cases in which all three tests were in accord in their negative readings and is of no value for purposes of comparison. There were 1,884 patients in this group.

Group B.—All tests positive. All cases in this group were considered syphilitic by full clinical confirmation. The patients numbered 483 and when added to the following group gave us a basis for comparing sensitivity (see Table III).

Group C.—With one or more tests negative or doubtful (serologic discord). Investigation of the 163 cases in this group revealed in each instance an undoubted syphilitic infection so diagnosed either through previous or subsequent serologic studies or through clinical evidence. The majority of these cases came from our syphilis clinic and represented in the large majority cases under treatment. The data in connection with Group C are shown in Table II.

Group D.—Certainly nonsyphilitic. This group included only individuals who were carefully and extensively studied or were intelligent volunteers familiar with the object of this study and who had every reason to believe themselves free from infection. This group constituted 200 individuals and all gave negative results to the three tests performed. This class is interesting only on conforming the specificity of all three tests.

COMMENT

The data indicating specificity of the three tests are tabulated in Table I; the cases with serologic disagreement, arranged as to clinical types of syphilis, are summarized in Table II. It will be noted that few if any early untreated syphilis cases fall in this category. It has been shown many times previously that the three tests individually are entirely adequate in detecting early infection. It is in latent and late cases where positive serology is most important in diagnosis that we most frequently encounter serologic conflicts. Of growing importance is the serologic progress of the patient under treatment. The progress in any case will be influenced to a great extent by the sensitivity of the test or combination of tests used as a criterion.

TABLE I

A COMPARISON OF THE SPECIFICITY AND SENSITIVITY OF THE WASSERMANN (KOLMER), KAHN AND EAGLE TESTS

	WASSERMANN TEST (KOLMER)						KAHN TEST			EAGLE TEST		
	0.2 C.C. OF SERUM			0.1 C.C. OF SERUM								
	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.
Certainly syphilis (Groups B and C)	546	21	79	516	17	113	531	34	81	621	2	23
Certainly nonsyphilitic (Group D)	0	0	200	0	0	200	0	0	200	0	1	199

TABLE II

163 CASES OF SYPHILIS IN WHICH THE SEROLOGIC TESTS WERE NOT IN AGREEMENT (GROUP C)

TEST	CONGENITAL			EARLY (TREATED)			LATE (UNTREATED)			LATE (TREATED)			TOTAL		
	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.
Wassermann 0.1 c.c.	2	3	14	0	1	11	5	2	4	26	11	84	33	17	113
Wassermann 0.2 c.c.	6	2	11	1	3	8	8	0	3	48	16	57	63	21	79
Kahn test	5	4	10	4	5	3	1	3	7	38	22	61	48	34	81
Eagle test	16	0	3	10	0	2	9	1	1	103	1	17	138	2	23

TABLE III

A COMPARISON OF THE SENSITIVITY OF THE WASSERMANN (KOLMER), KAHN AND EAGLE TESTS WHEN USED ALONE AND IN COMBINATIONS. (RESULTS OBTAINED WITH 646 SYPHILITIC SERUMS FROM PATIENTS INCLUDED IN GROUPS B AND C)

	POSITIVE		DOUBTFUL		NEGATIVE	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Wassermann 0.2 c.c.–Eagle–Kahn	634	98.1	12	1.8	0	0
Wassermann 0.1 c.c.–Eagle–Kahn	631	97.6	9	1.3	6	0.9
Wassermann 0.2 c.c.–Eagle	631	97.6	5	0.7	10	1.5
Wassermann 0.1 c.c.–Eagle	627	97.0	3	0.4	16	2.4
Eagle–Kahn	623	96.4	8	1.2	15	2.3
Eagle	621	96.1	2	0.3	23	3.5
Wassermann 0.2 c.c.–Kahn	583	90.2	28	4.3	35	5.4
Wassermann 0.1 c.c.–Kahn	569	88.0	25	3.8	52	8.0
Wassermann 0.2 c.c.	546	84.5	21	3.2	79	12.2
Kahn	531	82.1	34	5.2	81	12.5
Wassermann 0.1 c.c.	516	79.8	17	2.6	113	17.4

In Table III may be seen the relative sensitivity of the three tests under study, both alone and in combination, in the diagnosis of syphilis or the detection of antibodies in late and treated cases. As a single test the Eagle (96.1 per cent pos.) exceeded the Kolmer-Wassermann 0.2 c.c. (84.5 per cent pos.) and the Kahn (82.1 per cent pos.). The Kolmer-Wassermann, using 0.2 c.c. of serum as the maximum dose as recommended by Boerner and Lukens[1] and adopted by Kolmer,[5] gave 2.3 per cent more positives than the Kahn and 4.7 per cent positives than the same test using a maximum of 0.1 c.c. as originally recommended by Kolmer. The Kahn test gave 2.3 per cent more positives than the Kolmer-Wassermann with 0.1 c.c. as the maximum dose. It is to be noted that the Eagle test gave but 2 doubtfuls whereas the Kahn gave 34, the Wassermann with 0.2 c.c., 21 and with 0.1 c.c., 17. These tests should be given credit for not entirely missing these cases. It appears to us that the classification of these reactions as doubtful is due to the conservative methods of reading these tests rather than to the tests themselves. For this reason it may be fairest to the tests to compare the number of negatives which represents the number entirely missed. The Eagle test (3.5 per cent neg.) gave less negatives than the Wassermann 0.2 c.c. (12.2 per cent neg.) or the Kahn (12.5 per cent neg.). The Wassermann 0.2 c.c. and the Kahn are about equal, both giving about 5 per cent less negatives than the Wassermann 0.1 c.c. The relative sensitivity of the three tests remain about the same whether judged by the number of positives or negatives.

As might be expected, a combination of all three tests gave the greatest number of positive reactions. The showing of the Kolmer-Wassermann 0.2 c.c. and Eagle tests combined (97 per cent pos.) greatly exceeded the usually duplicated tests, Kolmer-Wassermann 0.1 c.c. and Kahn tests (88 per cent pos.), and gave 1.2 per cent more positives than the Eagle and Kahn combination.

The relative specificity of the three tests is shown in Table I. The Wassermann and Kahn with 200 certainly nonsyphilitic serums gave 100 per cent negative results, while the Eagle gave one doubtful and 99.5 per cent negative. Although this series is small it gives us a fair idea as to the chances of false positive reactions. All three tests failed to give a false positive reaction. The false doubtful reaction obtained with the Eagle test was repeated with the same

result thus eliminating the possibility of technical error. If we consider one false doubtful in 200, a serious offense then, it merely means that our interpretation of the Eagle test should be corrected to the extent that all reactions now read as doubtful should be classed as negative. This change would make it equal to the other tests as regards specificity without appreciable altering its high degree of sensitivity.

CONCLUSIONS

1. In a series of tests with 646 syphilitic serums employing the Kolmer-Wassermann, Kahn and Eagle tests, the Eagle test greatly exceeded the Wassermann or Kahn tests in the number of positive reactions.

2. The Eagle test alone proved more sensitive than the Wassermann and Kahn combined.

3. The Wassermann using 0.2 c.c. as the maximum dose was more sensitive than when 0.1 c.c. was used and about equal to the Kahn test.

4. The best combination of two tests was the Wassermann using 0.2 c.c. of serum and the Eagle test.

5. The Wassermann and Kahn tests failed to give a single false positive reaction with 200 serums from certainly nonsyphilitic individuals. The Eagle test gave one doubtful reaction.

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THE ESTIMATION OF THE PHOSPHOLIPID CONTENT OF THE WHITE BLOOD CELLS*

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IN A NUMBER of recent communications[1-7] variations in the lipid composition of the white blood cells have been reported in health and disease. The results of this series of investigations have served to emphasize two facts of prime importance: (a) leucocytoses which may be indistinguishable by microscopic examination of the blood may yet be entirely different by chemical analysis of the white blood cells. A search into the cause of this difference led to the discovery and establishment of the second fact that (b) active leucocytes in patients who recovered normally from fever, infection, surgical operations, or parturition possessed a lipid composition markedly different from that of inactive leucocytes in patients who succumbed to infection or in whom convalescence was prolonged.

Although each of the lipids shown to be present in the white blood cells was found to vary with activity of the blood leucocytes, the changes in one lipid stood out as most consistent and most extensive. This lipid was phospholipid and its relation to activity was found to be analogous to that reported by Bloor and his associates in studies of the physiology of glands and muscles and by Boyd in the ovaries[8] and the fetus and placenta[9] of rabbits. Briefly, it was shown that active leucocytes contain large percentages of phospholipid while inactive leucocytes contain much smaller amounts.

Having established this fact, the author has made use of phospholipid determinations on the white blood cells in arriving at an opinion as to the chances for recovery of patients referred to hospital with severe infections, marked febrile reactions, postoperative fevers, etc. From observations made to date, it would appear that in the absence of any potent toxin (as in tetanus) the following prognoses may be attached to phospholipid percentages in the blood leucocytes of *infected* cases:

500 mg. per cent or less	very poor
500-900 mg. per cent	poor to questionable
900-1,300 mg. per cent	fair to good
1,300-1,600 mg. per cent	very good
Over 1,600 mg. per cent	excellent

These delineations may require slight shifts in the light of further and more extensive investigations but they are offered from present experience with this method as guides for those who may desire to use it. The prognosis as so estimated is valid in those cases where recovery depends only upon the resistance of the body to infection. When some powerful toxin is present, the resistance

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to infection as calculated from the leucocytic phospholipid may be high but the patient succumbs to the effects of the toxin. For example, a case of incomplete abortion complicated by tetanus infection was found to have a leucocyte phospholipid of 1,400 mg. per cent but this patient died from tetanus intoxication a few days later.

Having found the determination of phospholipid in the white blood cells to be of prognostic value, it was considered advisable to report improvements in the technic of isolating the white cells from blood, to gather together various modifications adopted in the estimation of phospholipid, in brief to outline the method now used by the author along with data on the experimental variation or error in technic which may be expected with this procedure.

The amount of blood needed depends upon the white cell count. When this is under 10,000 cells per c.mm., 25 c.c. of blood are required. As the white count increases, progressively less blood is needed; at 20,000, about 10 to 15 c.c., etc. Since the effect of ingested food upon the phospholipid content of the white blood cells has not as yet been determined, it is advisable to obtain a fasting specimen. It is probable that meals do not have a pronounced effect since the author has often been unable to insist upon fasting blood and yet the prognosis determined from leucocyte analysis has been unappreciably affected.

Blood is collected in oxalate or citrate bottles and centrifuged at full speed for one-half to one hour. Thorough centrifuging aids in the cleaner separation of the leucocytes from the plasma above and the red blood cells below. About 2 feet of rubber tubing are attached to a 25 c.c. pipette and the free end of the rubber tubing held between the teeth. The plasma is then drawn off to within a quarter of an inch or so of the white cell layer, a little air drawn into the pipette, the rubber tube tightly clamped between the teeth and the plasma in the pipette discarded. Successive repetitions of this procedure remove most of the plasma and the last trace is absorbed upon a strip of filter paper carefully edged down the side of the centrifuge tube.

A pair of wide tissue forceps are then introduced into the centrifuge tube, and it is occasionally possible to remove all of the white cells in one adherent piece. The forceps should not be closed too tightly over the leucocytes. The removed white cells are then placed on a watch glass, which has been previously cleaned in sulphuric acid-potassium dichromate cleaning mixture and thoroughly washed, dried, and weighed. As many as possible of the red cells and plasma are removed from the edges of the white cells on the watch glass with strips of filter paper. In this manner, what the author has termed the "leucocyte fraction" is isolated and weighed. Invariably it contains traces of red cells and plasma as impurities but analysis of this fraction approximates very closely the analysis of pure leucocytes[5] and use of this fraction has the distinct advantage of requiring much less blood than is needed if the cells are washed. Also it is possible that washing might affect the percentage of lipids present in the white blood cells.

When the leucocytes cannot be removed in toto as one piece, a slightly different procedure requiring some practice is resorted to. The tips of the tissue forceps are opened and dipped slightly below the leucocyte layer, beginning at one edge. They are then drawn up slowly, gradually bringing the ends of the

forceps almost but not quite together. A small globule of the white cells will thus be caught between the ends of the tissue forceps, and it is placed upon the weighed watch glass. This procedure is repeated until all of the leucocyte layer has been removed. In the majority of cases, this is the manner in which the white cells will have to be separated from blood.

After weighing (usually 0.15 to 0.2 gm. obtained), the leucocytes are covered with 2 to 5 c.c. of sand (alcohol-extracted, ignited sea sand) which is mixed with the cells to form a semidry mass. This prevents the white cells from clumping when they are next transferred quantitatively with a cleaned metal spatula to a 125 c.c. Erlenmeyer flask. Roughly 50 c.c. of alcohol-ether (3:1, both redistilled and the ether peroxide free after Bloor[10]) are added and the flask immediately and thoroughly shaken. Small portions of alcohol-ether are placed on the watch glass, the residue of adherent cells completely worked off with the spatula and these washings added to the Erlenmeyer flask. The contents of the flask are then brought to boiling for a few minutes on a steam bath, cooled, and filtered. If more than one lipid is to be estimated, the filtrate may be made up to volume and aliquots taken, but for phospholipid alone (or phospholipid and free cholesterol, the latter determined on the acetone mother liquor as below) the filtrate need not be made up to volume. It is advisable to use filter paper which has been extracted with alcohol in continuous extractors for twelve hours to remove any possible lipid material. The residue of sand and precipitated leucocyte protein is washed several times with small portions of alcohol-ether, collecting the washings with the original filtrate. All glassware used must be previously cleaned with chromic acid, thoroughly rinsed with tap water and distilled water and dried by draining.

The resulting alcohol-ether extract contains all of the lipids present in the leucocytes and some nonlipoidal material soluble in alcohol-ether. These impurities are removed by evaporating off the alcohol-ether and redissolving the lipids of the residue in petroleum ether which is a much more selective solvent for lipids than alcohol-ether. The procedure for determining phospholipid is based upon Bloor's method of 1929[11] with modifications at various times introduced by Boyd.[1, 12, 13]

The alcohol-ether extract contained in a 100 c.c. tall beaker is evaporated almost to dryness on an electric hot plate using a glass rod with an unglazed end to prevent bumping. The beaker and contents are then transferred to the steam bath where all the solvent is evaporated off at a temperature of about 80° C. Then 2 to 3 c.c. of petroleum ether (redistilled after standing over concentrated sulphuric acid at least twenty-four hours) are added, brought to a gentle boil on the steam bath and the resulting solution of lipids poured off into a cleaned graduated 15 c.c. centrifuge tube. This is repeated 4 times with small portions of petroleum ether to make a final volume of 10 to 12 c.c. in the centrifuge tube. Ordinarily none of the thin layer on the bottom of the beaker will dislodge but if any flakes or visible material are carried over into the petroleum ether solution in the centrifuge tube, the latter must be centrifuged and the clear supernatant solution transferred quantitatively to another tube.

A cleaned, dry glass rod of $\frac{3}{16}$ inch diameter is placed in the centrifuge tube and the petroleum ether evaporated to a volume of somewhat less than 1

c.c. by immersing the lower half inch of the tube in about a quarter of an inch of boiling water contained in a beaker. The glass rod effectively prevents bumping by forming a small pocket of volatilized solvent in the conical bottom of the centrifuge tube. The glass rod is then removed and washed into the tube with a very small amount of petroleum ether. The petroleum ether solution of the lipids is then made up to 1 c.c.

The phospholipids are precipitated from this solution by adding 7 c.c. of dried (calcium chloride) redistilled acetone followed by 0.1 c.c. of 30 per cent magnesium chloride in 95 per cent alcohol, mixing and allowing one-half an hour for complete precipitation. They are then centrifuged out and the acetone mother liquor poured off, being cautious not to dislodge any particles of the precipitate. The precipitate is washed once or twice with 2 c.c. of dried acetone, centrifuging and decanting each time. The tube is then inverted to drain, dried with a gentle stream of air and the precipitate dissolved in 10 c.c. of ether (redistilled and peroxide-free) which is saturated with distilled water (a layer of distilled water is kept in the bottom of this ether reagent bottle). The precipitate is dislodged with a finely pointed glass rod, the whole stirred several times during an interval of fifteen minutes or more allowed for solution of the phospholipids. A whitish material is left undissolved which consists mostly of magnesium chloride together with some diaminophospholipid[13] and cerebroside, if the latter be present. This material is centrifuged out and the supernatant solution of the phospholipids drawn over by suction through a glass tube into a 125 c.c. glass stoppered Erlenmeyer flask (oxidation flask). The glass tube is rinsed several times with ether, the rinsings being caught in the oxidation flask. The ether is then evaporated off at a low temperature on the steam bath, the last traces of ether vapor removed with a gentle stream of air and the phospholipids are left as a thin scum on the bottom of the oxidation flask. Once all the ether has been evaporated off, the flasks should not be left on the steam bath, else the phospholipids may be partially oxidized and low readings obtained.

The quantity of phospholipid thus isolated is determined by oxidation, 1 mg. of phospholipid requiring 3 c.c. of 0.1 N potassium dichromate to completely oxidize it.[1] Oxidation is carried out by adding to the oxidation flask exactly 5 c.c. of 2 per cent silver chromate in concentrated sulphuric acid (prepared after Bloor[11]) followed by exactly 3 c.c. of 1 N potassium dichromate. The flask is shaken, loosely stoppered and placed on a cast iron plate, ½ inch thick, in an electric oven maintained at 124° C. where it is heated for twenty minutes. It is not necessary to open the oven until oxidation is completed if the flasks are thoroughly shaken before being put in.

On removal from the oven, further oxidation and destruction of reagent are prevented by adding 50 to 75 c.c. of ice cold distilled water, the flasks stoppered and set aside until ready to titrate. They are titrated with 0.1 N sodium thiosulphate after adding 10 c.c. of 10 per cent potassium iodide (freshly prepared), using a few drops of 1 per cent starch emulsion as an indicator.

If all reagents are pure and glassware clean, a blank need not be introduced until the stage where the silver chromate reagent and potassium dichromate are added. Here a blank glass stoppered Erlenmeyer flask is introduced and carried through with the sample. The titration of the sample subtracted from the titra-

tion of the blank gives the c.c. of 0.1 N thiosulphate, and by equivalent weights of 0.1 N potassium dichromate, due to the phospholipid present (usually 5 to 10 c.c. differences are obtained). The thiosulphate should be prepared some days or weeks prior to use and standardized at least every two or three weeks, especially if deteriorating rapidly. The titration difference, calculated in terms of 0.1 N potassium dichromate, is then divided by 3, then by the weight (in gm.) of the leucocytes extracted and multiplied by 100. The result indicates the number of milligrams of phospholipid present in 100 gm. of white blood cells.

The whole procedure may be readily completed in half a day and does not require the continuous attention of the analyst. There are several stages at which, if desired, the procedure may be interrupted for some time. These are (a) after alcohol-ether is added to the leucocyte-sand mixture; (b) when the extract is prepared and corked in a cleaned bottle; (c) at the stage of acetone-magnesium chloride precipitation and (d) moist ether solution of the phospholipids, at both stages by tightly corking the tubes; (e) after the moist ether solution is in the oxidation flask and (f) after cooling the oxidation mixture with ice water and stoppering. At Stages a and b the procedure may be held for weeks or even months; at Stages c, d, and e overnight and at Stage f for not more than a few hours. The procedure may thus be conveniently fitted into a research or routine laboratory time-table. When samples are allowed to collect at Stage b as many as 24 or 32 (multiples of 8 to fit the usual heavy duty centrifuge) may be conveniently analyzed in one day. It is not advisable to put more than 8 to 10 flasks in the oven at one time but the load of any oven may be found by trial. One blank will serve for each batch put into the oven or if heating is uniform, for all samples in which the same oxidizing reagents are used.

The above procedure has been repeatedly found by the author to give an experimental error of less than 10 per cent, the error in the phospholipid estimation itself being about 5 per cent (percentage standard deviation). For example, a sample of 400 c.c. of human blood was divided into 8 portions of 50 c.c. each. Each portion of blood was subjected to the above procedure from beginning to end. The resulting values for phospholipid in the white blood cells were: 1,080, 1,210, 1,230, 1,260, 1,300, 1,330, 1,340, and 1,460 mg. per cent, respectively, giving a mean of 1,276 and a standard deviation of 115 which is 9.1 per cent of the mean. Clinically, variations from one patient to another and from time to time in the same patient are usually much greater than this and up to several hundred per cent where physiologic or pathologic changes have taken place. The author considers a variation over 15 per cent in the same patient as suggestive and significant but very seldom is it necessary to consider experimental error at all as the changes are usually quite beyond it.

Finally, for comparative purposes, the phospholipid content of the white blood cells in normal individuals may be noted. Eight cases have been previously reported[1] as 457, 572, 643, 672, 747, 990, 1,164, and 1,168 mg. per cent, respectively. To these may be added the following determined at various times since: 505, 631, 747, 780, 820, 827, 868, 891, 905, 905, 920, 1,020, 1,040, and 1,290 mg. per cent, respectively. The mean of these 22 normal individuals is 844 mg. per cent with a standard deviation of 241 or 24 per cent of the mean. From these figures it may be calculated that statistically any value for phospho-

lipid in the white blood cells below approximately 600 mg. per cent and above approximately 1,100 mg. per cent will be outside the range which includes two-thirds of all values expected for normal individuals. Or further, 95 per cent of all values for phospholipid in the white blood cells of normal persons may be expected to lie within the approximate range of 400 to 1,400 mg. per cent.

Although such figures arrived at by statistical analysis are significant in connection with the expected variation among normal persons, clinically more information is obtained from repeated analyses on the same patient. A rising curve for leucocytic phospholipid indicates increasing resistance to infection and a falling curve decreasing resistance to infection. The significance of single determinations in patients *already infected* may be judged from the range of values given in the earlier part of this communication.

SUMMARY

The author's method for separating the white blood cells from blood and determining their phospholipid content has been described.

This procedure has been shown to admit an experimental error of less than 10 per cent.

The phospholipid content of the white blood cells of 22 normal persons was found to be 844 mg. per cent on the average with a standard deviation of 241.

The significance of single and of repeated readings in the interpretation of prognosis in the course of infections has been outlined.

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A RESPIRATORY CHAMBER FOR PRODUCING ANOXEMIA IN MAN*

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IN 1915, Kolls and Loevenhart[1] described a respiratory chamber for small animals. A modification of this chamber has been in use in our laboratory for the past several years, in connection with our studies on anoxemia, in which animals were used. However, in the course of these studies a point was reached at which it became desirable to make certain observations on human subjects. A special respiratory chamber was constructed for the purpose of producing anoxemic conditions in man. This chamber has also been used for animals too large to be placed in the smaller chamber mentioned above.

The chamber is constructed of 5.0 mm. boiler plate steel, and all seams are oxy-acetylene welded. It is cylindrical in shape, one end being concave, the other end convex. The outside dimensions are: 42 inches in diameter and 73 inches in length. The long axis is placed in the horizontal plane. The size is sufficient to accommodate comfortably two average adults, in either a sitting or a recumbent posture. When sitting, the occupants place their backs against the concave end of the chamber. As will be noted from the diagrams and the subsequent description this does not interfere with the passage of air either to or from the chamber. Two windows are placed in the chamber, one on either side. These provide light for the interior, and serve as a means of communication between the occupants and the operator of the chamber. The windows are made of 12 mm. plate glass, mounted in heavy cast iron frames (*K*). A circle of glass, 7¾ inches in diameter, is exposed in each window. The construction of the entire chamber is such that it is capable of withstanding complete evacuation. Fig. 1 shows a top view of the entire chamber, while Fig. 2 is of a sagittal section through the long axis.

The door (*D*) of the chamber is oval in shape, and is placed in the center of the top. The opening measures 18 by 24 inches. The door and its facing (*F*) are made of heavy cast iron. The rim of the door rests on a 1½ inch shelf of the facing, and both of these surfaces are machined so as to make an air-tight joint when fitted with a rubber gasket (*G*) ⅛ inch thick. Fig. 2 includes a vertical section of the door, and reference is made to this figure in the following description.

In order to seal the opening the door is put in place and the arms (*A*) are rotated so as to be directly under the catches (*C*). The wheel (*H*) is then given a few left hand turns. This carries the casting (*B*), and with it the arms (*A*) upward on the shaft (*J*), causing the arms to impinge under the catches. The upward pressure thus exerted on the catches is transferred to the door as downward pressure, through the arms and the shaft. Hence, forcible loosening of

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the wheel exerts downward pressure on the door, and seals the crevices between the rim of the door and the gasket on the shelf of the facing. Likewise, in removing the door, a few right hand turns of the wheel release the arms from underneath the catches. The casting (*B*) can then be rotated until the arms are free of the catches and the door can be lifted off the opening.

A 1½ inch galvanized iron pipe (*P*) leads from the center of the convex end of the chamber to a Crowell rotary air pump, Type 4D. This pump is run by a 5 horse power electric motor, and has a capacity of 40.5 cubic feet of air per minute, which insures excellent ventilation for the occupants of the chamber. The pump and motor can be housed at some remote spot, wherever space is available. In our installation this machinery is housed in the basement of the building (see Fig. 4).

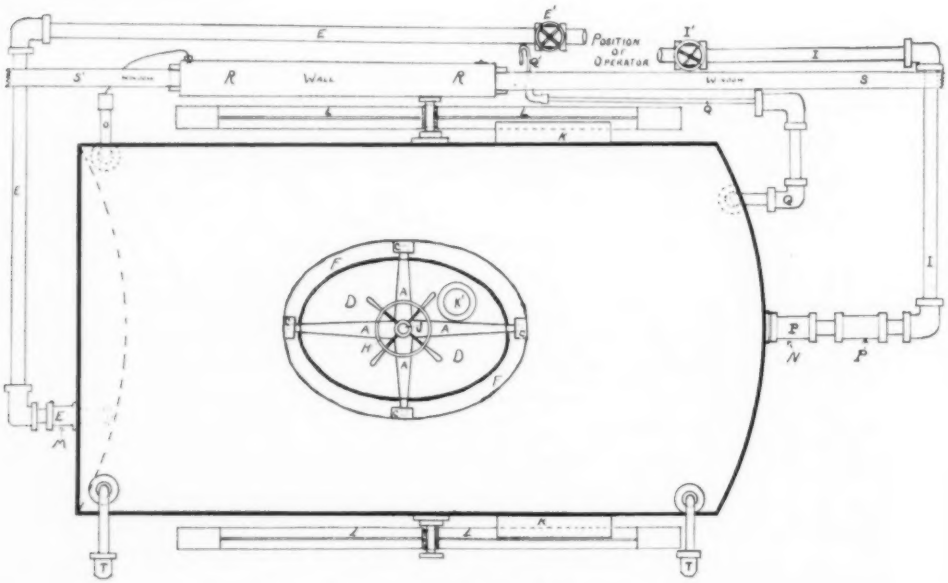

Fig. 1.—Top view of respiratory chamber.

Air is allowed to enter the chamber through a 1½ inch pipe (*E*), inserted in an upper lateral quadrant of the concave end. This position was chosen so that air might be drawn the entire length of the chamber, and further so that the occupants might assume a sitting posture without interfering with the entrance of air. This pipe is provided with an ordinary steam valve (*E'*), so that the amount of air entering the chamber is subject to easy control. There is a similar valve (*I'*), opening to the outside, in the pipe leading from the chamber to the pump. The purpose of this valve is to divert part of the suction produced by the pump, allowing the chamber to be decompressed slowly, so as to avoid any discomfort to the occupants.

The chamber is connected by means of a ¾ inch iron pipe (*Q*) and appropriate reducers, with a mercury manometer (*Q'*) attached to a meter stick, so that the total pressure within the chamber can easily be measured. As an added

precautionary measure, a barometer is placed within the chamber, and is arranged so as to be visible to the operator on the outside. This eliminates the danger which might arise should a leak develop in the manometer apparatus.

Electrical current is led into the chamber through a pipe (*O*), so fitted as to make an air-tight seal around the insulated wire. This current is used to light the interior of the chamber, and to provide heat on cold days. It has been found that a fan is unnecessary, since a considerable stream of air constantly passes through the chamber.

Two safety valves (*T*) are placed in the wall of the chamber. They open to the outside, and serve as added protection for the occupants. The action of these valves is exactly opposite to that of a pressure safety valve; that is, they allow air to enter the chamber at any predetermined degree of decompression.

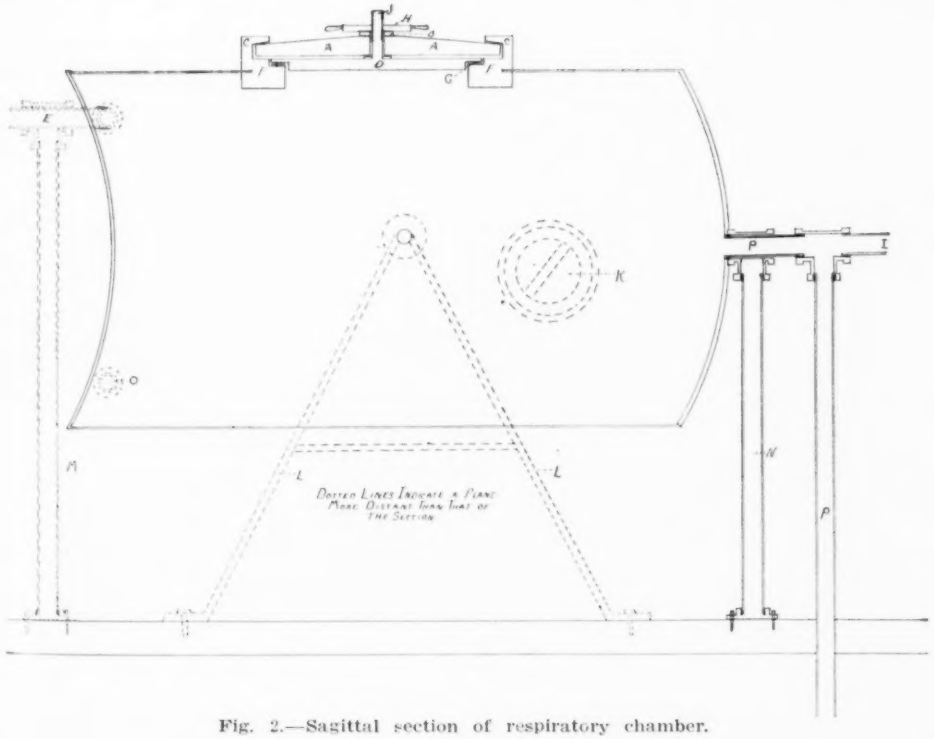

Fig. 2.—Sagittal section of respiratory chamber.

In order to conserve space the chamber may be placed outside the building (*R*), convenient to either a door or a window (*S-S'*). The inlet pipe (*E*) may enter the building, and the valve (*I'*) on the pipe (*P*) to the pump may also be placed within the building by an extension of pipe (*I*). The manometer (*Q'*) may be placed against the inside wall beside the valves, as may the switch controlling the pump motor. Thus may the operator be located indoors conveniently and comfortably, yet be in constant communication with the subjects in the chamber, through the windows. Fig. 1 gives an idea of this arrangement.

In our installation the chamber is placed just outside two windows on the ground floor. It is supported by two sets of legs (*L*), and is steadied by braces (*M*) and (*N*), respectively. It might be noted here that the diagrams in Figs.

1 and 2 do not correspond exactly with the photograph in Fig. 3. This is because the diagrams show the way the chamber should be arranged in an ideal installation. A wooden ladder or stairway may lead from the ground level to the top of the chamber for convenience in gaining access to the door.

The principle of producing anoxemia is quite simple. Air is drawn from the chamber faster than it is allowed to enter. In operating the chamber the following procedure is followed:

The subjects are placed in the chamber, the door is put in place and closed as described above. The air pump is then started, with all valves open. Valve (I') is then slowly closed, and decompression of the chamber begins. The proper degree of decompression can then be reached and maintained by properly setting valve (E'). It was found that a firmly seated steam valve would maintain

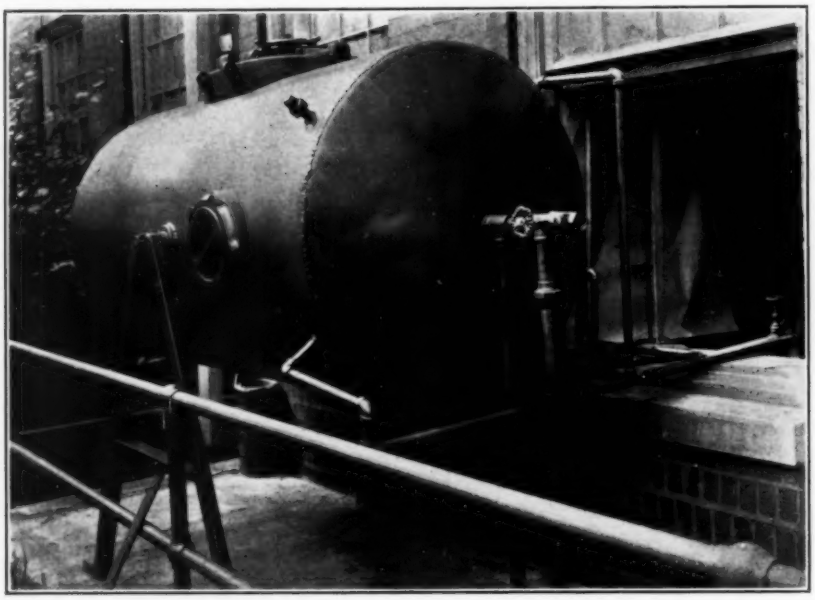

Fig. 3.

a constant setting, and since the speed of the pump remains constant, any predetermined degree of decompression can be maintained within the chamber, simply by setting valve (E') at the proper point.

When it is desired to remove the subjects from the chamber, this procedure is reversed. That is, valve (E'), then valve (I') is slowly opened, allowing the pressure within the chamber to return to the normal atmospheric level. The pump is then stopped and the door is removed.

It will be seen that the safety of the subjects has been given primary consideration in the construction of this chamber. It has given very satisfactory service, and is easy to operate. The only trouble encountered by any of the subjects was that of a little difficulty in adjusting the ears to the changes in pressure. In general, it was found that a rate of decompression equivalent to

an ascent of about 2,000 feet per minute could be maintained without discomfort to the average person. The pressure could be allowed to return to normal at a similar rate.

This particular chamber is a rather new addition to the equipment of this laboratory. However, for the past several years investigations upon the physiologic effects of anoxemia have been carried out in this laboratory, using a chamber identical with this one, except smaller in size.

In 1933 Van Liere, et al.[2] reported that anoxemia delayed the emptying time of the stomach in dogs, and that the delay could be elicited at an oxygen percentage of 15.35 per cent, corresponding to an altitude of only 8,000 feet, a very mild degree of anoxemia. At an oxygen percentage of 9.4 per cent, corresponding to an altitude of about 20,000 feet, the emptying time was in some instances increased to 400 per cent of its normal.

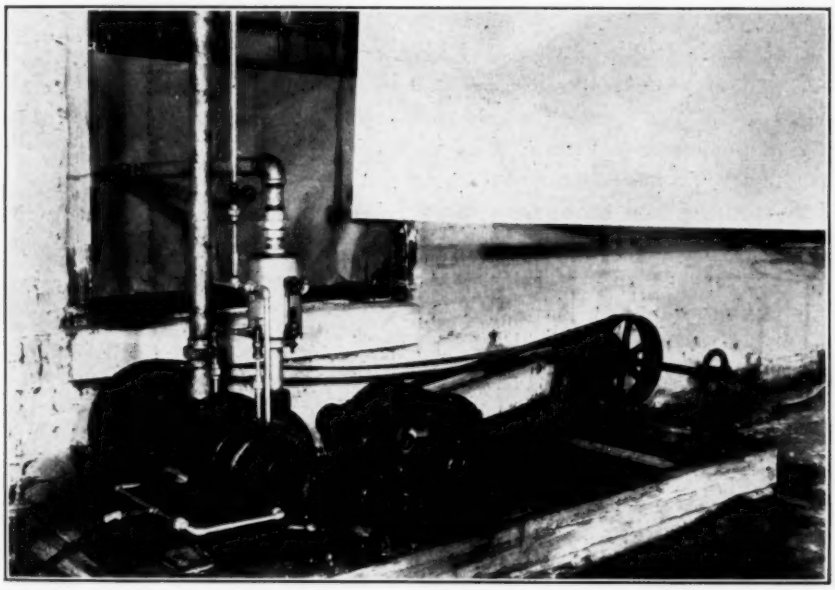

Fig. 4.

Further work was carried out by Crisler, Van Liere, and Wiles[3] in an attempt to determine the mechanism involved in the delayed emptying time. It was found that the delay at mild degrees of anoxemia was brought about by a pylorospasm of vagospastic origin, while higher degrees of anoxemia produced, in addition, a depression of the activity of the gastric musculature.

Of course the field of gastric motility is only one of the many fields of use that such a chamber as the one described might find in a physiology laboratory, for work already reported leads one to believe that the effects of anoxemia must be manifold.[4-6] Our purpose in installing the large chamber was to check certain of these findings in human subjects, as well as to pursue any new investigations desired.

It must be mentioned that the chamber described herewith has a very wide field of applicability. In addition to its use for the production of partial

vacuum it can be converted, by a few simple changes in the pump, into a pressure chamber. Such a pressure arrangement could be used either for experimental investigation or in therapeutics, as has been pointed out in the literature. Further, since it can be made gas tight, the chamber could be used for studying the effects of definite concentrations of various gases upon any animal organism. The value of the chamber is enhanced still further by the fact that, by a few simple additions, it would be possible to control, within narrow limits, the temperature within it.

It is quite possible that such a chamber, which can be subjected to rapid changes in pressure, would be of value in ear work; the opinion of an otologist would be of interest upon this point. The size of the apparatus, of course, would limit its use here.

The chief value of such a chamber lies, perhaps, in the fact that much research should be done upon the condition of anoxemia, owing to the popularity of airplane travel. The latter is becoming more and more important, and many individuals each year are subjected to a new physiologic environment. Commercial air transport companies find that it is best to fly at an altitude of 10,000 to 12,000 feet. A report of the Air Medical Service (1919) states that unacclimated persons are often sick at an altitude of 10,000 feet, and that many persons never become accustomed to an altitude of 14,000 feet. This would certainly indicate that much more work is needed upon the physiologic changes in anoxemia.

In summary: The principles of construction and operation of an apparatus by which human or large animal subjects can be subjected to reduced pressures are described. Some of the physiologic effects of lowered oxygen tension are mentioned, and other possible applications of such a chamber are pointed out.

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A LABORATORY ELECTRIC FURNACE SUITABLE FOR MICRO-KJELDAHL DIGESTIONS AND SIMILAR USES*

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PHILADELPHIA, PA.

AFTER experiencing difficulties due to draughts and insulating deposits of soot when using gas heating for micro-Kjeldahl digestions, attention was turned to electrical heating. The lack of suitable standard equipment made it necessary to design and construct a furnace especially for the purpose. A battery of four electric furnaces (enabling 24 digestions to be performed simultaneously) of the type described below was, therefore, devised. The furnaces are easy to assemble and dismantle (should the necessity of changing the heating coils ever arise), and have the heating coils entirely protected from draughts. They have proved to be very satisfactory, and embody a sufficient number of features of importance to suggest that a description of the assembly and its use will be of value to other workers.

Materials of Construction—

¼ in. asbestos board.
Sheet tin.
¾ in. pine or deal board.
4-way (off-low-medium-high) reciprocating oven switch.
Nichrome wire, for heating units. (Alloy V, 22 B. & S., resistance 1 ohm/ft. Driver Harris Co.)
½ in. asbestos tape.
1 in. flat-headed nails.

Description.—The sides, bottom and ends of the furnace are made of two thicknesses of the asbestos board, the inner pieces being cut smaller as required for proper fitting. This portion of the furnace is rigidly nailed together. The top of the furnace consists of one thickness of asbestos board, piereed by six 1-inch diameter holes (approx. 2¼ inches from center to center), and is kept in position by the sheet tin covering bent to cover the sides, bottom, and part of the top (see Fig. 1). The tin covering is kept in position by a tin binding strap (1½ inches in width) bent so that ¾ inch fits over the ends. A single nail, at the joining of the tin binding, is sufficient to keep the whole in position. The carrier for the heating units consists of a single thickness of asbestos board fitting loosely into the furnace, and which is nailed to transverse supports of asbestos board at each end and in two intermediate positions. If not adequately supported the carrier will sag, due to the heat, causing the coils to touch and short-circuit. The coils contain 20 feet of the nichrome wire which, before being stretched between the terminals at the ends of the furnace, was wound to a coil approximately ½ inch in diameter and 4 inches in length. The terminals are ordinary ⅛ inch diameter brass bolts and nuts.

*From the Cancer Research Laboratories, Graduate School of Medicine, University of Pennsylvania.
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The assembled furnace is mounted on pieces of the asbestos board resting on ¾ inch thick wooden strips screwed to the baseboard, and is held firmly in place by two ½ inch tin straps (screwed to the mounting) under which runs asbestos tape for heat insulation. A separate wiring diagram is not given since the actual connections will vary with the switch used. For high heat, the heating coils are connected in parallel; for medium, one coil alone is used, and for low, the two coils are connected in series. The two terminals at the end of the furnace not shown in the sketch are permanently connected by a short piece of ½ inch brass strip, and one of these terminals is also connected to the black wire running between the two furnaces (see Fig. 1). By dividing the lead-in wires as indicated, one connection to the 110 volt a.c. supply serves two furnaces while still maintaining the independent operation of each. Ordinary, 18-gauge, twisted lamp cord is used for the wiring of the furnace.

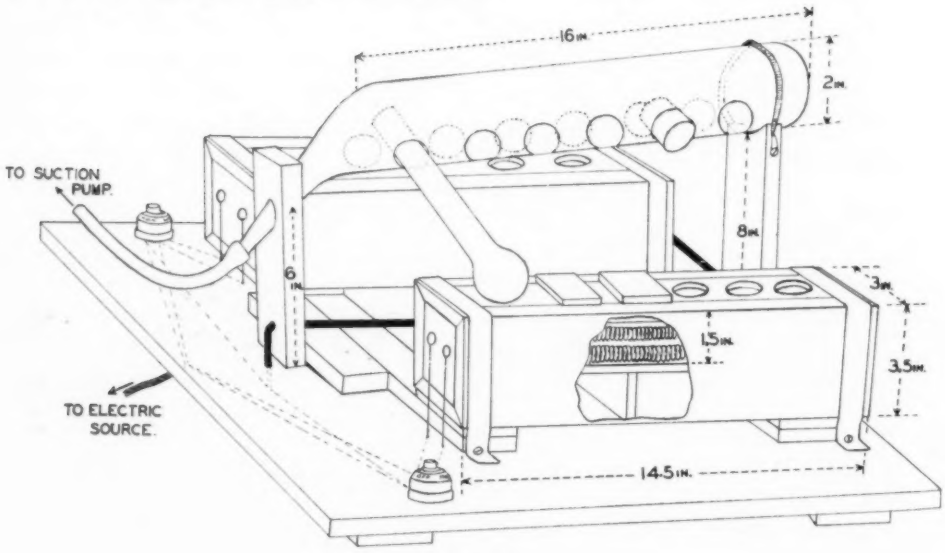

Fig. 1.

The exhaust system consists of a 2 inch diameter Pyrex glass tube closed at one end and drawn out at the other, and pierced with holes (of slightly greater diameter than the neck of the flasks used), at suitable positions relative to the holes in the furnace tops. The two furnaces emptying into the same exhaust are slightly staggered, as are the two sets of holes in the exhaust tube, so that the necks of the flasks being heated on the one furnace fall, in the exhaust tube, between those of the flasks on the companion furnace. The exhaust tube is mounted at an angle for drainage purposes, as shown, the closed end being secured by means of a spring passing around it, and is connected to a water suction pump. The heating apertures not in use are conveniently covered with small pieces of asbestos board, and the corresponding exhaust holes by means of corks.

One of us (E. B. S.) has used a battery of four such furnaces for Kjeldahl digestions almost daily for the past four months without any breakdown or accident, all four being frequently used simultaneously for seven or eight hours

continuously. They have proved to be very rapid, a total nitrogen microdigestion, using 0.1 c.c. of plasma, taking two to three hours on the average. No trouble has been experienced either with bumping or with the cracking of flasks due to condensate running down the outside of the digestion flasks.

The rubber tubings from the two exhaust tubes of the four furnaces in use at present are connected by a Y-tube to a trap for the condensate, a wash-bottle filled with concentrated sodium hydroxide solution (for further protection of the filter pump) and finally to a water suction pump. This has been found to remove satisfactorily all the fumes from the digestions, even though the apparatus is used on a bench in the open laboratory. The micro-Kjeldahl flasks employed are very satisfactory, being made from 18.0 ± 0.5 mm. outside diameter Pyrex tubing, 1.2 mm. wall thickness, with a spherical bulb approximately 1.5 inches in diameter, the flasks having an over-all length of about 7 inches.

A NEW METHOD AND A NEW PIPETTE FOR BLOOD SEDIMENTATION*

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A NEW blood sedimentation method with a new pipette is described in this paper. It consists of using a straight glass tube, or pipette, of small uniform bore, by first filling it with an anticoagulant solution, and then blowing it out, leaving a thin film of the anticoagulant lining the inside or bore of the tube. Then the tube or pipette is filled by allowing the blood to flow into the pipette directly from the blood vessel of the patient, or else by sucking in the blood from a drop formed by pricking the finger, or any other suitable area of the body.

Then the pipette is set up in the vertical position, and the sedimentation of the red blood corpuscles is observed and recorded and plotted on a suitable chart.

The pipette consists essentially of a straight glass tube of about 1 to 1.5 mm. bore. The pipette has a ground glass tip at one end, which fits into a hypodermic needle. At the other end of the glass pipette a short piece of rubber tubing is attached, which at the free end has a mouthpiece to be used for sucking fluid into, or blowing it out of the pipette. The pipette is divided into two unequal parts by a stopcock which cuts the lumen of the pipette precisely 100 mm. from the extreme end of the ground glass tip; but cuts the lumen of the shorter limb about 30 mm. from the end to which the rubber tube is to be attached. The longer limb of the pipette is graduated into millimeters, with zero at the extreme end of the ground glass tip,† and with the 100 mark exactly at the point where the lumen of the graduated limb is cut by the stopcock.

*From the Louisiana State University Medical Center.
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†Though the zero point is at the extreme end of the tip, there are no graduations marked on the tip because the engraving would weaken the tip too much.

In making the measurements of blood sedimentation rate, the following steps are performed as directed below:

1. First prepare a suitable anticoagulant solution, such as a sterile solution of 20 per cent potassium oxalate, put up in a sterile condition in a rubber capped 10 c.c. vial.

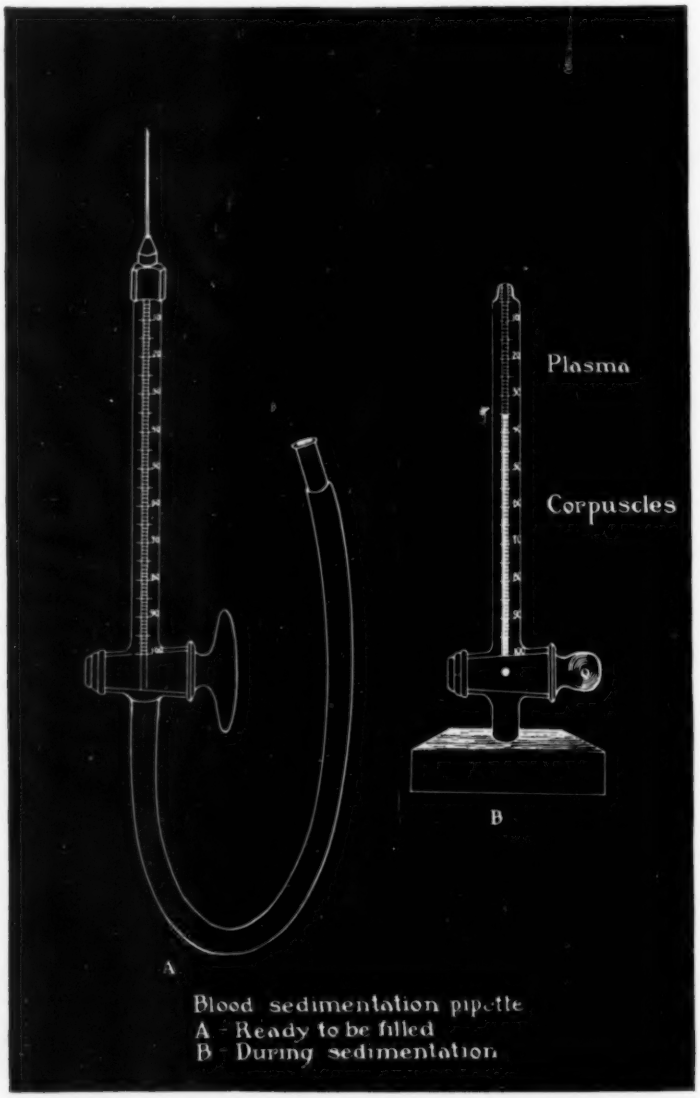

Fig. 1.

2. Sterilize a blood sedimentation pipette, hypodermic needle which will fit the ground glass tip of the pipette, and also a short piece of rubber tubing with mouthpiece.

3. Adjust and fit the needle to the tip of the pipette, and attach the rubber tube with mouthpiece to the other end of the pipette.

4. Next sterilize the surface of the rubber cap of the vial of 20 per cent oxalate solution. Insert the needle which is attached to the tip of the pipette. Now, with the cock open, suck the oxalate solution through the needle and up into the bore of the pipette, filling it completely as far as, or a little beyond the 100 mark. Now turn the cock closing the lumen of the pipette, withdraw the needle from the vial of oxalate solution, and lay the filled pipette down on some sterile gauze.

5. Next prepare the patient's arm: adjust a rubber band around the patient's arm, rub some antiseptic over the skin covering the veins at the inner side of the bend of the elbow.

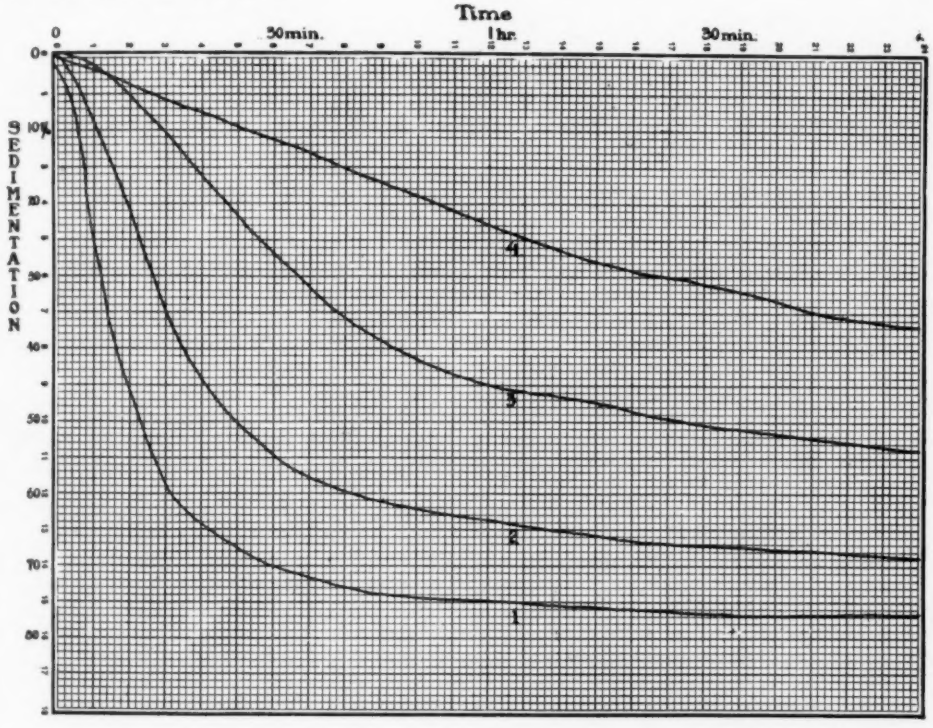

Fig. 2.—Series of four sedimentation curves, representing four different stages of the same case of severe lobar pneumonia of the left lung. *1*, Extremely rapid sedimentation curve during the stage of complete consolidation. The patient is extremely ill. *2*, Improved sedimentation curve (less rapid), during the stage of resolution. The patient is much improved. *3*, Further improved sedimentation curve, toward the end of resolution. The patient is about to leave the hospital, although there is still an area of solid lung (unresolved) in the lower part of the left lung. *4*, Normal sedimentation curve after the patient has completely recovered. Both lungs are clear.

6. Now open the cock of the pipette and blow out the oxalate solution, leaving a thin film covering the inside of the pipette. Then quickly insert the needle into the lumen of the vein and allow the blood to flow up through the needle into the lumen of the pipette, filling it completely as far as the 100 mark or a little beyond that point.

7. Next close the cock and withdraw the needle from the vein.

8. Turn the tip of the pipette upward in the vertical position, and, with a bit of gauze, remove the needle from the tip and wipe off the ground glass tip, leaving the upper meniscus of column of blood flush with the end of the tip.

This leaves the pipette completely and precisely filled from the very mouth of the tip (zero point) to the point where the stopcock cuts the lumen of the pipette (100 mark). Thus a column of fresh blood is cut off and held in the pipette, which column of blood is precisely 100 mm. in length. This measurement is automatically effected by closing the cock and removing the needle and wiping the tip.

9. Next immediately set the pipette up in a holder with the tip pointing vertically upward, and begin to observe the settling of red corpuscles, reading off the settling on the scale* and reading the time from a stop watch. Observe the settling, say every minute in rapidly settling cases, and every five minutes in slower ones. Keep up the observations for two hours. Record the results and plot the curves on coordinate paper.

In this method the blood is allowed to flow directly from the blood vessel of the patient into the pipette, where it is immediately set up and the observations of sedimentation at once begun. This means there is no dilution or mixing of the blood and no settling of the blood corpuscles before the actual observations have been started.

The stopcock automatically cuts off the column of blood precisely at the 100 mark and the removal of the hypodermic needle leaves the upper meniscus of the column exactly at the mouth of the tip of the pipette, which means the column of blood is exactly 100 mm. long. This measurement, being automatic, is always uniform and precise even in the hands of inexpert laboratory workers. With this method the results of measurements or rate of blood sedimentation are so accurate that very slight alterations in blood sedimentation can be determined with certainty. By sterilizing the pipette and needle and rubber tube before filling the pipette, the observations can be continued for twenty-four hours or longer in order to determine the more complete sedimentation reading, without any decomposition occurring in the blood.

There is no possibility of leakage because the stopcock effectively closes the lower end of the graduated portion of the pipette. No air can get into the lumen of the pipette because the blood flows into the pipette under its own pressure. If the needle does not fit the ground glass tip quite nicely, there may be some blood leaking out; but no air will pass into the pipette and no air bubbles can get mixed with the blood.

Since the graduations on the barrel of the pipette are in millimeters, and since the graduated portion of the barrel is 100 mm. long, the graduations represent, not only millimeters, but they also represent percentage of settling of the red blood corpuscles. This is a convenience in making and calculating the readings and in plotting the curves showing the results.

The whole procedure is so simple and easy to perform and so precise in results, that it should be generally adopted and used by the medical profession.

*Since there are no graduations engraved on the ground glass tip, the measurements of settling in this part of the pipette must be read by either estimating the readings or by placing a millimeter scale beside the ground glass tip.

BOCK'S ERYTHROCYTOMETER AND TECHNIC FOR ITS USE*

E. A. SHARP, M.D., AND E. M. SCHLEICHER, A.B., DETROIT, MICH.

BOCK[1] in 1933 described an inexpensive erythrocytometer employing the well-known principle of halometry. This method for measuring blood cells is so well known through the work of Pijper, Eve, and others that comment is unnecessary.

Inasmuch as the Metz-Echelon micrometer eyepiece (Leitz) was under study at the time Bock described his instrument, a comparative study of these two methods of erythrocytometry appeared attractive.

It will be seen by the diagram (Fig. 1) that Bock's halometer is compact and simple in construction. It consists of three sections: the table (*A*) is superimposed on the outer supporting tube (*B*); within the main supporting tube is a movable, close-fitting cylinder (*C*) controlled by a set screw (*J*) which can be released or secured during the mensural procedure; the third section serves as the supporting base (*E*) and lighting apparatus (*F* and *G*). The table has a central aperture (*K*) 1 cm. in diameter. The movable inner cylinder (*C*) is closed by a diaphragm at its lower end. Dual apertures (*I*) in the diaphragm, each 0.5 cm. in diameter, permit the two beams of light to pass through the object of study placed over the central aperture on the table. The light in the base is transmitted through a frosted glass plate (*H*).

The set screw, attached to the movable inner cylinder through a slot in the outer envelope, is used to control its vertical excursion within the outer envelope. On the opposite side from the set screw is another vertical slot in the outer envelope through which projects a pointer (*D*) attached to the inner sleeve. The pointer moves over a scale calibrated in 0.2 microns. The over-all height of the apparatus is 38 cm.

Preparation of the Blood Film.—In order to make erythrocytic observations uniform a standard blood smear is employed. The objective of the technic is to prepare the "margin-free" smear of Schilling. The blood is taken from a fingertip, which is wiped off sharply with ether (not alcohol) to make it hyperemic. The skin is punctured about 5 mm. deep with a lancet (7 mm. long and 3 mm. in width, Eimer & Amend, New York). The first drop oozing from the puncture, or upon very slight pressure, is discarded with the aid of a clean gauze sponge. The second small drop is picked up with the edge of a No. 2 coverslip, 18 mm. size, the specimen edge of the coverslip being placed about one-half inch from one end of the microslide at an angle of 45°. After allowing the blood to spread along the edge of the coverslip it is drawn toward the other end of the slide with

*From the Anemia Laboratory, Out-Patient Department, Harper Hospital.
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an even, firm and rapid movement, so that the blood follows without any crushing. A satisfactory preparation is made from a drop of blood that will make a smear extending one-third to one-half the length of the slide.

A "usable area" in the standard, unstained blood film is selected by microscopic examination. It is determined by the appearance of an area where the cell edges are touching or almost touching each other without any appreciable overlapping.

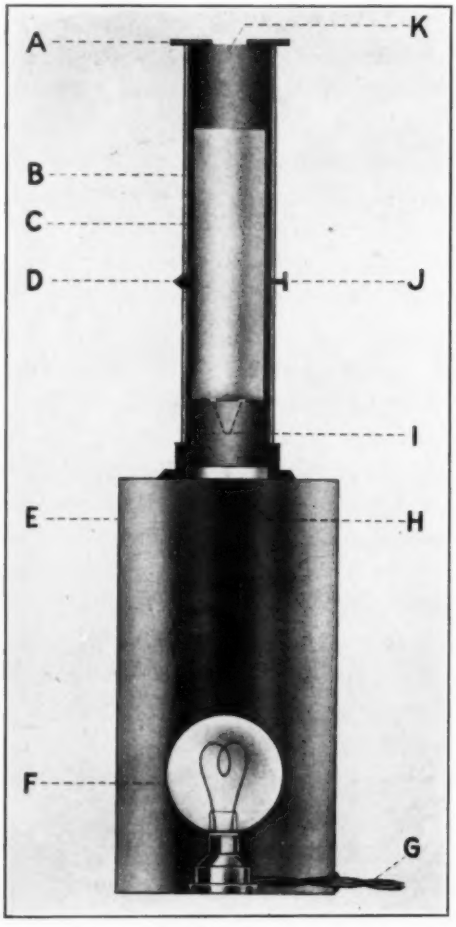

Fig. 1.

The Determination of Mean Diameter of Erythrocytes by Bock's Halometer.—When a "usable area" has been found in the unstained blood preparation, the microslide is placed on the table of the erythrocytometer, film side up, so that the "usable area" completely covers the aperture. An unstained blood film on a high-grade glass slide shows two distinct, sharply defined, spectral halos within the movable cylinder. The outer red bands are used for measurement. A black line should not be present between the two red halos at the reading point, nor should there be any difference in the intensity of the colors of the contiguous red bands. By manipulating the set screw, lifting and depressing the

inner sleeve, two well-defined red halos are brought together without overlapping, the set screw is secured and the measurement is taken from the scale.

To insure greater accuracy of reading, record the initial measurement. loosen the set screw, depress the inner sleeve slowly until two yellow bands touch each other, then slowly elevate the sleeve until the peripheries of the red bands are again in apposition. This procedure can be repeated by elevating the sleeve to "gray" and lowering to the "red" again.

Two blood preparations are always used for halometry. Four readings on each film are taken from their respective usable areas. The grand total of the readings in microns divided by eight represents the mean diameter of approximately one million erythrocytes.

Range of Mean Diameters in Anemia.—The mean diameter, determined by Bock's erythrocytometer on 44 relapsed cases of pernicious anemia, has ranged from 7.9 to 9.0 microns. Compared with mean diameters on the same cases determined on 200 red cells measured by the Echelon ocular micrometer the difference between the two calculations has been negligible. There is, however, a great difference in the time required for the two procedures. The mean diameter can be determined with a Bock instrument in two to three minutes, while the measurement of 200 cells with the ocular micrometer is well known as a time-consuming procedure.

It is noteworthy that there was no instance when the halometric mean diameter was at variance with the determination made by the ocular micrometer in the course of studying several thousand blood films. One exception may be when extreme degrees of hypochromemia were present. Faint halos result from deficient erythrocytic pigment; hence, experience is required to insure accuracy in extreme hypochromasia.

A comparative study of stained and unstained preparations discloses that there is a slight difference in mean diameters taken on the unstained and the stained blood film, seldom more than 0.5 microns has been detected. The lower reading is found in the stained preparation.

It is believed, therefore, that Bock's erythrocytometer is a satisfactory instrument for the rapid determination of the mean diameter of red blood cells. Comprehensive data on comparative mean erythrocytic diameters determined by the halometer and ocular micrometer on various anemias are being organized for a later communication.

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A GENERAL PURPOSE POLYCHROME BLOOD STAIN*

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THERE have been many excellent blood stains published and some of them, such as Jenner's,[1] Wright's,[2] and Giemsa's,[3] widely used in the recent decades. All of these and most of the other good ones are in reality modifications of Nocht's[4] and Jenner's[1] stains or embody the fundamental principles thereof. Romanowsky[5] in 1891 had published some studies of the malarial organisms in which he seems to have been the first to use a mixture of eosin and methylene blue. Nocht[4] in 1898 mixed Unna's polychrome methylene blue with eosin to obtain a mixture which was the first truly polychrome stain, but he did not know he had made new chemical compounds and did not separate the eosinates from the mixture. Jenner[1] in 1899 was the first to filter off the precipitate obtained by mixing aqueous solutions of eosin and methylene blue and to dissolve it in methyl alcohol for use as a blood stain. The May-Grünwald[6] stain of 1902 is to all intents and purposes the same as Jenner's.[1] The Wright stain which is so universally used, and Hasting stain, which is practically identical with it, and other variants, named and unnamed, are modifications of the Leishmann[7] stain. These Leishmann variants differ only in the manner in which the methylene blue is heated with a dilute alkali and the kind of alkali and the amount of heat used to form oxidation products. Giemsa thought to obtain some of the fractions of the eosinated polychrome methylene blue mixtures and recombine them in fixed proportions. The stain is good so far as it goes but he did not realize how many fractions there were. He made clear the importance of a methylene azure, fixing in the literature the words "azure stain," "azure granules," "azure chromatin," etc.; but he did not know there were at least three methylene azures and probably several more similar compounds to which reference will be made later. Some of the now less utilized staining mixtures, like Pappenheim's universal, were Jenner's or May-Grünwald combined with some one of the several Giemsa formulas. Kehrmann[8] in 1906 was the first to throw some real light on the chemistry and the complexity of the blood stains, and MacNeal[9] in 1922 in his tetrachrome stain did put some of these facts together into a stain formula. Conn[10] in his discussion of this stain sequence will be found most illuminating and was the source of some of these facts. The only reason for reporting another staining mixture would be to shorten the manufacturing process, simplify the technic of staining, improve the results, or for adaptation to some special use. The stain which I now report does somewhat simplify manufacturing and staining technic. It gives uniformly good results and has done so in various hands over a period of some twenty years. Recently it has been put to particular use in the photomicrography of blood.

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It seems to be well adapted to both black and white and natural color processes. The technical procedure and particular application of the stain in photomicrography will be given separately.

This stain has become rather widely used locally and as its use has been spread by graduates of the College of Medicine, Syracuse University, and internes, numerous requests for the formula have been received.

The so-called polychrome feature of the present-day stains is a complex thing, by no means dependent upon any single feature. The basic commercial stains are but mixtures, particularly the blues and violets, and the variations of the eosins cover a considerable range. When one makes a so-called eosinate of methylene blue, he makes more than one eosinate; he not only makes several eosinates of methylene blue as chemical entities, but also makes eosinates of other basic dyes present in small amounts in the original methylene blue. Other variations are dependent upon the eosin used. The same is true of eosinates of methylene azure, methyl blue, methyl violet, and thionin. I became impressed with this fact through working with the various Giemsa stains and modifications. A reasonably pure eosinate of methylene azure giving certain very definite and well-known so-called azure qualities in nuclei and chromatin will lack some of the other characteristics of a so-called panoptic stain. It would seem therefore that various combinations of the reasonably pure eosinates of methylene azure with other eosinates would produce a good general purpose stain. In the very generally used Wright's stain and in Hasting's stain and others of the Leishmann type, solutions of methylene blue are heated with a weak alkali to produce uncertain amounts of methylene azure. In some manner the alkali is then washed out or neutralized and an eosinate made. I became convinced that the variability in different lots of stain was due in part to slight changes in pH and in the amount of electrolyte present due to alkalization and neutralization. The workings of all textile and paper makers' staining methods suggest the importance of the electrolytes and colloidal dispersion in staining qualities and penetration. Hayden's blood method using a buffered dilution solution is a valuable contribution based on these principles. The method I devised for making stain took into account that we were dealing with mixtures to start with and that the more the blues were mixed within reasonable limits, and the more carefully one avoided the use of alkali or other additions which might disturb the ultimate balance, the better and more uniform the results would be. The necessary amounts of the various secondary constituents are small (Wilson[11]). To laboriously and expensively separate and "purify" them only to recombine them is a waste of time and money. They can be obtained in reasonable amounts by mixing various commercial types. What is more, additional minor but useful and beautiful characteristics are unexpectedly obtained. The stain to be described takes advantage of these facts. Its manufacture and the technic of its use are both simplified. An extensive experience with students and technicians has shown it to be a sound routine stain. It is nearer foolproof than the more complicated ones and rather uniformly good results may be obtained by the unskilled. When most carefully used I believe extremely good results may be expected.

Only minor changes in the formula for this stain have been made over a fifteen-year period of use. The present formula is a revision to meet the new

and more definite standards of the Committee on Standardization of Biological Stains. It has been thoroughly tested in its present form and used routinely for over two years, and we are convinced our manufacturing results are more uniform and the staining results have improved since using this stain.

Groat's Modification of Jenner's Stain

No. 1		No. 2	
Eosin yellowish		Methylene Blue U.S.P. medicinal	
Water and alcohol sol.		C. I. No. 922*	
C. I. No. 768*		Total dye content 88 per cent	5.0 gm.
Total dye content 94 per cent	6.0 gm.	Methylviolet 2B	
Distilled water	500 c.c.	C. I. No. 680*	
		Total dye content 81 per cent	1.0 gm.
		Thionin (Erlich-Hoyer)	
		C. I. No. 920*	0.2 gm.
		Distilled water	500 c.c.

*Certified by Commission on Standardization of Biological Stains, H. J. Conn, Chairman. National Aniline & Chemical Corporation certified stains were used by us.

Mix Solutions 1 and 2 and warm gently (use asbestos) to 50° C. and let stand twenty-four hours in incubator. Filter using hard filter paper, wash residue three times with cold distilled water. The last water should be pale blue. Dry thoroughly. Pulverize off paper.

Stain.—

0.5 gm. powder
100 c.c. Absolute Methyl Alcohol (chemically pure, free from acetone)
Stand two weeks, shake frequently.

The staining period is best determined by staining a normal smear. Improves with age if shaken occasionally. However, use only the clear supernatant fluid or filtrate.

METHOD OF USE

For a Single Slide.—Cover slide as fully as possible with undiluted stain and stain for an average of five minutes. Then plunge slide and stain thereon into 50 c.c. of neutral distilled water, mix stain and water by agitating the slide and then allow slide to rest vertically in the mixture of stain and water until it becomes rose pink. For coverslip spreads use proportionate amounts.

For Routine Laboratory Use.—Coplin jars may be used with pure stain in the first, diluted 1 to 10 stain in second, and third jar of clear, or nearly clear distilled water for final "pinking" of slightly overstained slides. Average time five minutes in first, five minutes in second, and rinse in third jar.

For Photography.—Purplish tints are required. Use fresh and filtered stains and dilutions, six minutes in first jar, fifteen minutes or more in the second jar (1-10 dilution) and quick rinsing in third jar (neutral distilled water) or until pink.

Stains vary in time required but wide latitude in routine work gives good results. For best effects slightly overstaining in the first jar with considerable prolongation of time in the second and possibly the third jars is recommended. Water freshly distilled once from permutit softened supply is recommended.

For Marrow.—Direct thin spreads of marrow from trephine biopsies or fresh autopsy material are made on slides. These are covered with pure dry ether for three minutes, again with fresh ether for three minutes and then with 95 per cent ethyl alcohol for three minutes and dried in air. They may then be stained as for blood for examination and for photography. Increase staining period 25 per cent or more if spreads are thick.

We hold with those who believe that marrow aspirations are more or less mixtures of circulating blood and marrow. However, smears made from such material may be stained like blood or marrow depending upon the character of the mixture.

When a marrow method involving emulsification of a bit of marrow in clear human serum is used, the spreads may be stained as blood, increasing the staining time unless very thin.

When this stain is used routinely the nuclei of the polymorphonuclear leucocytes are quite densely and clearly blue with fine detail. The nuclei of the large lymphocytes are pale purplish blue and of the smaller lymphocytes deep purplish blue. The nuclei of the monocytes are grayish blue with a distinct, fine reticulum as contrasted with the coarsely lobulated and wavy chromatin pattern of the lymphocytes. Nuclei of normoblasts are bluish black, and of the younger erythroblasts variable paler shades of blue with good detail of the chromatin pattern depending upon maturity as in other stains. The neutrophile granules are a bright rose pink and stand out prominently. A rather remarkable variation in granule size and brilliancy brings out the various stages of maturity, the younger ones being more distinctly defined, varying from purplish to rose. The so-called toxic reaction is well defined with blue granules very clearly shown. The neutrophile granules stand out more sharply than they do with an average Leishmann type stain. Eosinophile granules are slightly but definitely brighter and may be distinguished by their larger size. Basophile granules are purple. Fine dustlike, blue gray granules appear in mature monocytes. The so-called azure granules in the various mononucleated cells are well shown. Cytoplasm of leucocytes varies in practically the same manner as in Wright's and other similar stains. Malarial parasites are shown quite definitely greenish blue with sharply defined chromatin of a rich pink to magenta. Auer bodies, Schüffner's dots and Cabot's rings are shown as in other panoptic stains and blood platelets are lavender with magenta chromatin. Erythrocytes are a bright rose pink. Polychromatophilia is very well shown, both the diffuse and the punctate. Basophilia of lead poisoning is clearly shown, the granules being a bright blue.

With more careful manipulation as described for photography, the fine staining of the granules can be maintained and at the same time various shades of purple may be emphasized in the lymphocytes and made to appear in the polymorphonuclear nuclei to the extent desirable for photography, while the nuclei of the monocytes remain more definitely bluish gray and erythroblast nuclei bluish black. All the good staining features of the red cells are maintained. Bluish, greenish, or washed-out erythrocyte effects are avoidable in staining for parasites and platelets.

The comparative ease with which the stain may be made and used is indicated by the fact that at first we made the stain at a central laboratory and distributed it, but for some ten years now at the Medical College and at the Syracuse hospitals where this stain is used as a routine, the various technicians are making equally good batches of stain and have only minor difficulties in handling it. Internes take to it readily and continue to use it after they leave us. As already said, the good results obtained with it in photomicrography and the technical reasons for them will be given in a separate paper embodying also some of the technical features of blood photomicrography.

COMMENTS

Present knowledge of the chemistry of blood stains leaves much to be explained. There are three eosinates of methylene blue, at least three thionin azures, one or two methylviolets and undoubtedly numerous other eosinates in the mixtures commonly used. "Polychrome," rather than "trichrome" or "tetrachrome," is more descriptive for present-day blood stains.

The physical state, colloidal balance, and hydrogen ion concentration are potent factors in all staining and dyeing processes. With the best chemical and technical control, actual results cannot be wholly predicted. The textile does not match; the paper is mottled instead of uniform tint; the blood cells are "off color"; and often none can tell why.

The manufacturing method herewith described corrals as many useful ingredients as possible without undue expense, the product seems to have a sufficiency of them, and we avoid the known pitfalls of application and consolidate much of the valued experience of others into simple procedures.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ANEMIA of Premature Infants, Abt, A. F. Am. J. Dis. Child. 49: 1204, 1935.

In the series of premature infants here reported on, the values for whole blood iron closely approximated the values for hemoglobin.

On comparing the curves for the whole blood iron and the hemoglobin values in the series of premature infants with similar curves for full-term infants within the first eight months of life, it was found that the values are equally high at birth and the rates of fall in the two groups are parallel; the lowest values are reached at about the twelfth week of life. The types of the curves are similar, the chief difference being that in premature infants the hemoglobin and the blood iron values reach lower levels at corresponding age periods.

The factor

$$\frac{\text{Hemoglobin in percentage}}{\text{Whole blood iron in mg. per hundred c.c.}}$$

determined by Kotikoff for full-term infants is practically the same as that here reported for premature infants.

It has been noted that certain common factors produce the anemia of the full-term as well as that of the premature infant during the early months of life. These are the change from a low intrauterine oxygen tension to the higher postnatal tension of atmospheric air; the increased hemolysis of erythrocytes after birth; the fragility of the immature red blood cells of the newborn, and the embryonal type of hemoglobin, different chemically and spectroscopically from the hemoglobin of older infants.

The premature infant differs, in part at least, from the full-term infant because he has a relatively more rapid growth, and he should therefore have a more rapidly increasing blood volume with a greater demand for formation of blood; a more immature and probably less efficient hematopoietic system (functional incapacity), and a smaller supply of blood to begin with, which might be expected to yield a more limited amount of blood-building materials. The greater decline in the values for hemoglobin and for whole blood iron in the premature infant as compared with those in the full-term infant may be explained by these physiologic factors which are peculiar to prematurity.

SYPHILIS, and Pregnancy, a Clinical Study of 2,150 Cases, Mc Cord, J. R. J. A. M. A. 105: 89, 1935.

Pregnancy does not affect the reliability of the Wassermann reaction.

This test should be a routine part of antepartum care.

Regardless of the activity of the disease, sufficient antepartum antisyphilitic treatment assures the woman a syphilis-free baby in 95 per cent of the cases.

The best results will be obtained with ten or more treatments.

Treatment should be mild but continuous and should not be controlled by the Wassermann reaction.

The concurrent use of arsenic and a heavy metal has worked well.

Such therapy seems to be safe for the mother.

In the vast majority of cases, a strongly positive cord Wassermann test, properly done, means that the baby has congenital syphilis. A negative cord Wassermann reaction is of little value in the diagnosis of congenital syphilis.

The characteristic picture of osteochondritis of the long bones is pathognomonic of congenital syphilis.

There seems to be no condition in medicine that returns such huge dividends in life and health with such a small output of energy and money as that seen in the prevention of congenital syphilis.

LEUKEMIA: Its Diagnosis and Treatment, Rosenthal, N. J. A. M. A. **104:** 702, 1935.

The relative occurrence of the three principal groups of leucemia apparently corresponds with the relative percentage of the various types of leucocytes in the circulating blood: namely, granulocytes, lymphocytes, and monocytes.

The underlying systemic disorders present in all cases is essentially the same. An arbitrary division may be made according to the duration of the disease, acute or chronic, and also according to the number of white blood cells, into leucopenic and leucocythemic forms.

Symptomatology, although of great value in differentiating the disease, is unreliable for purposes of diagnosis. This should be based on the characteristic blood changes, which do not depend so much on the number of white blood cells as on the presence and persistence of specific types of cells, such as myelocytes, myeloblasts and relative and absolute lymphocytosis.

Confirmatory diagnosis of the more obscure varieties may be made by biopsy on the sternal bone marrow or on a lymph node.

The treatment of leucemia is largely symptomatic. Arsenic, transfusions and particularly roentgen irradiations are the chief means of inducing symptomatic improvement, remission, or possibly prolongation of life.

LEUKEMIA: Differential Diagnosis of the Leukemic States, Kracke, R. R., and Garver, H. J. A. M. A. **104:** 697, 1935.

There still remains no unanimity of opinion relative to the origin of white blood cells, but it seems probable that under normal conditions myeloid cells arise mainly from the bone marrow and lymphoid cells from the lymphoid glandular system, including the spleen.

It is virtually impossible to distinguish between the various types of leucoblasts with ordinary staining methods, or even with vital staining.

Cases of chronic leucemias can be diagnosed easily as well as acute forms, provided the total white cell count is definitely increased with a preponderance of blast cells.

The leucemic states in which the total number of white cells is normal, or below normal, offer considerable difficulty in diagnosis both clinically and hematologically and are likely to be confused with various leucopenic diseases.

The most reliable criterion for the diagnosis of any leucemia is a preponderance of immature cells regardless of the total number. Studies on a large number of cases of monocytic leucemia that are filed in the Hematological Registry indicate that the chief cell type has its origin in the bone marrow and adds further evidence that monocytic leucemia is but an atypical phase of myelogenous leucemia.

MYELOMA, Bence-Jones Proteinemia in Multiple, Cantarow, A. Am. J. M. Sc. **189:** 425, 1935.

A case is reported of multiple myeloma (granulocytic type) with severe anemia and renal failure. Bence-Jones protein was present in the urine and was also demonstrated in the blood serum.

The total serum protein concentration varied between 6.84 and 11.3 gm. per 100 c.c., the Bence-Jones protein being approximately 3 gm. when the total protein was 8.7 gm. and 5.2 gm. when the latter was 11.3 gm. per 100 c.c.

Hypercalcemia (12.9 mg. per cent) was present at the time of admission, the serum calcium concentration falling to 9.2 mg. per cent while the serum inorganic phosphorus concentration increased from 5.8 mg. to 12.5 mg. and the blood creatinine from 6.1 mg. to 13.6 mg. per 100 c.c.

The relatively rapid and marked fluctuations in serum protein and serum calcium concentrations in patients with multiple myeloma and the variability of the several factors which determine the characteristic precipitation reactions of Bence-Jones protein emphasize the necessity for repeated studies in such cases.

TUMORS, Malignant Epithelial, of the Neck, Oliver, R. L. Am. J. Cancer 23: 16, 1935.

A study of 80 cases of carcinoma arising in the deep tissues of the neck without relation to the epidermis or glandular organs is presented. The relationship of these tumors to the development of the branchial clefts is discussed, and the embryology of these structures, which form the basis for the origin of the tumors, is outlined.

Among the 80 cases, males predominated in the ratio of nine to one, and the peak of age incidence was in the sixth decade. The average duration of symptoms was between six and seven months. The usual symptom of onset was the appearance of a tumor in the neck without relationship to clinical findings in the throat or in other organs in the cervical region. Stiffness of the neck, headache, hoarseness, pain, and general cervical node enlargement were among the other clinical findings. In about 10 per cent of the cases trauma marked the onset of the clinical history. The position of the tumor was usually in the upper anterior cervical triangle, behind and below the angle of the jaw.

Only radical surgery sufficed as an attack upon these rapidly growing and infiltrating tumors. The usual operation advocated involves the block dissection of the glands of the neck with resection of the larger vessels of the neck, including in some instances the internal and common carotid artery.

Pathologically the tumors were grossly either solid or cystic; under the microscope they could be divided into squamous, cuboidal, and basal-cell forms. The cuboidal and basal-cell forms were subdivided into two groups: diffuse and alveolar.

In the differential diagnosis it was found necessary to rule out cervical lymphadenitis, benign branchial cleft cysts, malignant lymphomas, and metastatic carcinoma from the nasal sinuses or structures of the throat.

A consideration of the ultimate results in relation to the modes of treatment and the types of cellular pathology justifies the conclusion that these tumors, which are usually fatal (77 out of 80 cases), are best treated by radical surgery if seen in the earlier stages. They comprise a single pathologic entity grading from the less differentiated basal-cell forms through the cuboidal-cell type to the most highly differentiated squamous cell form. Surgery is slightly more favorable in these last two forms than in the first. Irradiation is probably advisable in conjunction with surgery in all forms, but particularly in the basal-cell type. In advanced and inoperable cases palliative irradiation may be helpful.

TISSUE: Preservation and Microscopic Examination of Nodules in Gout, DeGalantha, E. Am. J. Clin. Path. 5: 159, 1935.

1. Fix fresh material immediately in absolute alcohol. Two changes of four hours each for thin specimens; longer (to twenty-four hours) for thicker specimens.
2. Xylol paraffin (equal parts xylol and parawax kept at 57°) for two hours.
3. Hard paraffin (melting point 56°) for one hour and embed.
4. Cut sections about 8 microns thick, fix to slide and dry in oven at 37° C. for two hours.
5. Remove paraffin with xylol and then wash in absolute alcohol.
6. Stain three minutes in Harris hematoxylin.
7. Wash in absolute alcohol and counterstain with 2 per cent alcoholic eosin one minute.
8. Clear in several changes absolute alcohol, carbol xylol and xylol.
9. Mount in xylol Canada balsam.

For specific demonstration of urate crystals, use above technic through stage 5; then:

6A. Place slides in 20 per cent silver nitrate solution and expose to strong sunlight until the urates are a bright rose color (one to four hours).

7A. Prepare solution of (a) 10 c.c. 3 per cent gelatin in hot water; (b) 3 c.c. 20 per cent silver nitrate and (c) 2 c.c. 2 per cent hydrochinon, and pour this over slides until the urates turn black and the connective tissues are yellow.

8A. Wash quickly in hot water (57° C.).

9A. Clear in absolute alcohol and xylol as in stage 8 above.

10A. Mount in xylol Canada balsam.

For decalcifying specimens containing bone: (1) Place thin specimens in absolute alcohol containing 2 per cent concentrated nitric acid. Change to fresh solution every twenty-four hours, until the bone is softened. (2) Wash in several changes of absolute alcohol for twenty-four hours. (3) Embed and handle as above.

BLOOD CALCIUM, Clinical Estimation and Significance of, Mc Lean, F. C., and Hastings, A. B. Am. J. M. Sc. **189**: 601, 1935.

The total calcium of the serum or plasma is nearly all accounted for as calcium ions and calcium bound to protein. Of these two forms the ionized calcium is of primary physiologic and clinical importance.

The calcium ion concentration of the plasma is normally maintained within a relatively narrow range by a process of physiologic regulation in which the parathyroid glands play a prominent part. This range has been found, as a rule, to be from 4.25 to 5.25 mg. per 100 c.c.

The calcium ion concentration of the plasma, at any one time, is the resultant of an equilibrium between the total calcium and total protein present in the plasma.

A method for clinical estimation of calcium ion concentrations, by calculation from total protein and total calcium concentrations, is presented. Results obtained by this method have been found to be in substantial agreement with the results obtained by direct observation.

Fluctuations in the concentration of total calcium, occurring in response to fluctuations in the concentration of total protein in the plasma, are necessary for the maintenance of normal calcium ion concentrations, and consequently are not in themselves of clinical importance.

An increase in the calcium ion concentration in the plasma is presumptive evidence of hyperfunction of the parathyroid glands.

A decrease in the calcium ion concentration in the plasma may occur as the result of hypofunction of the parathyroid glands, or may be brought about by the hyperphosphatemia of uremia.

In all other conditions investigated, including various disorders of calcification, the calcium ion concentration of the serum or plasma has been found to be within normal limits.

BLOOD GLUCOSE CLEARANCE, Determination by a Microinterval Method. I. Studies in Normal and Diabetic Persons, Mc Kean, R. M., Myers, G. B., and Von Der Heide, E. C. Am. J. M. Sc. **189**: 702, 1935.

The authors feel that a "normal" microinterval curve (peak of 175 mg. per cent or below and fifteen-minute level of 125 mg. per cent or below) is strong presumptive evidence against the presence of diabetes mellitus. On the other hand, an abnormal curve is not of equal diagnostic significance. Curves similar to those obtained in diabetes have been found in several other conditions, such as cardiac decompensation, hypertension, cholecystitis, peptic ulcer, carcinoma and chronic encephalitis.

The fate of the injected glucose has been conjectured and an attempt has been made to explain the early appearance of a distinctive response in normals and diabetics.

PREGNANCY, Chemical Test for, Mencken, J. G. Deutsche med. Wchnschr. Leipzig 60: 1837, 1934.

Mencken found the test devised by Vischer and Bowman of value. The method follows:

Reagents.—(1) 0.5 per cent solution of hydrogen peroxide. (2) 1 per cent aqueous solution of phenylhydrazine hydrochloride. (3) 5 per cent aqueous solution of methyl cyanide. (4) Concentrated hydrochloric acid. The reagents should be freshly prepared each week.

Method.—(1) To 1 c.c. of urine add 1 drop of peroxide solution, 5 drops of phenylhydrazine solution, 5 drops of methyl cyanide solution, and 5 drops of HCl. (2) Place the tube in a boiling water-bath for twenty-five minutes.

Reading.—Positive: russet color and flocculent precipitate. Mencken usually obtained a dustlike and occasionally a somewhat more dispersed precipitate.

Negative: straw color and no or only a powdery precipitate. Mencken found negative reactions light and positive reactions dark in color.

PEPTIC ULCER, Gastric Mucin Treatment of, Fogelson, S. J. Arch. Int. Med. 58: 7, 1935.

A study based on questionnaires concerning 494 patients with peptic ulcer treated by clinicians throughout the United States has demonstrated the ability of gastric mucin to control all the symptoms in 70.5 per cent and to afford a partial relief of symptoms in 23 per cent, while failure to afford any relief occurred in 6.5 per cent.

In 217 patients with intractable ulcers who could not be relieved of symptoms by medical management, of whom 69 had been submitted to previous surgical procedures, gastric mucin afforded complete relief in 63.1 per cent, partial relief in 29.4 per cent, and no relief in 7.5 per cent.

The results obtained in this group of patients with intractable ulcers suggest the possibility of obtaining symptomatic relief with gastric mucin in a relatively high percentage of patients in whom accepted orthodox measures, including operation, have failed.

The permanence of the results is not considered, owing to the limited periods of observation.

TUBERCULOSIS, Renal, Spontaneous Healing of, Keyes, E. L. J. A. M. A. 104: 16:1380.

Medlar has shown that renal tuberculosis begins as a nonsurgical lesion that frequently heals.

This lesion may be identified clinically as a tuberculous bacilluria (as defined in the text).

Surgical renal tuberculosis, characterized by gross changes shown by pyelography, is clinically a progressive disease with a fatal termination unless interrupted by nephrectomy.

The pyelogram discloses surgical tuberculosis. The earlier the nephrectomy, the greater the probability of cure.

The healing of surgical renal tuberculosis by pathologic nephrectomy is extremely rare and, even with the kidney function gone, active tuberculosis may persist.

Though surgical renal tuberculosis may remain latent for long periods of time, latency is extremely rare and even in its most complete form, i.e., latency due to complete physiologic destruction of the kidney, the tuberculosis may still actually be active.

The curative treatment of renal tuberculosis, old and new, is nephrectomy.

WEIL'S DISEASE, Serological Diagnosis of, Brown, H. C. Brit. M. J. 1: 411, 1935.

The procedure following, the "adhesion test," has been found as reliable as the agglutination test in the diagnosis of infection with *L. ictohemorrhagicae.* It is also more rapidly performed and easier to read.

The agglutination test (Schuffner) is performed by allowing various concentrations of serum to interact with an equal volume of a young culture for two hours at 32° C. After incubation small drops from the various dilutions are placed on slides and examined, without a cover glass, by dark-field illumination.

The technic of the adhesion test follows:

The following reagents are required: (1) The patient's serum; (2) a young broth culture of *L. icterohaemorrhagicae;* (3) a saline suspension of a young culture of *B. coli* or other similar organism; (4) a fivefold dilution in saline of fresh guinea pig's serum (this is not required if the patient's serum is tested on the same day the sample is taken).

One volume (about 20 c.mm.) of each of the above reagents is placed in a small agglutination tube and the contents mixed; a control tube contains known normal serum in place of the patient's serum. The tubes are incubated at 37° C. for thirty minutes, and then a small drop is placed on a microscopic slide, covered with a coverslip, and examined by dark-field illumination. In the event of a negative reaction the leptospira will be seen swim-

ming freely and totally unimpeded by the presence of bacteria. In a positive reaction the bacteria will be seen to be firmly adherent to the leptospira. Not all the leptospira will be affected in this way, but only a certain proportion, unless the serum is of a very high titer.

At least twenty leptospira should be observed before a negative result is recorded. It may be that one or more bacteria, in a positive reaction, will adhere to the end of the organism, and no amount of movement on the part of the leptospira will dislodge them. Other leptospira will be seen almost completely covered with bacteria, with only the terminal portions still in sight. The reaction is very dramatic, and no difficulty should be experienced in reading the results.

What occasionally happens with a negative serum is that a leptospira may be seen more or less in the center of a small clump of bacteria. In this case it will be noticed that it is not adherent to the bacteria, but slides in and out of the bacterial clump, a totally different state of affairs from that in which there is true adhesion. When there is a positive reaction with the patient's serum a second test can be made, using dilutions of serum which will give total concentrations up to 1 in 30,000.

The reaction is marked by the end of the second week and may be expected to appear approximately as soon as the agglutination test (sixth day).

The culture medium used in the adhesion test is a modification of that recommended by Fletcher (1927), and is as follows: distilled water, 3 c.c.; lemco broth, 0.5 c.c.; inactivated rabbit's serum, 0.25 c.c. The culture is incubated at 32° C. for six days. It is preferable to use a strain which has lost its pathogenicity.

PNEUMONIA, Streptococcus, A Note on Parenteral Liver Therapy in, Wilson, J. A. Am. J. M. Sc. **189:** 374, 1935.

The injection of liver extract was tried in the first case of pneumonia because of the drop in leucocyte count from 16,000 to 8,200 in three days. When liver extract was started, this patient was seriously ill, in a light stupor, high fever, able to take very little nourishment, and voided 500 c.c. of urine in twenty-four hours. The prognosis seemed poor. Sixteen cubic centimeters of liver extract (equivalent of 800 gm. of fresh liver) were given intramuscularly between 4 P.M. of the fifth day and 4 P.M. of the sixth. The leucocyte count rose from 8,200 to 27,800. The patient improved. He voided 3,400 c.c. of urine in twenty-four hours, and although still confused mentally the stupor was less and the fever lower. The leucocytes remained about 20,000 until the tenth day of the illness. The fever came down by lysis and there were no complications. He was discharged from the hospital on the fifteenth day.

The leucocyte count in the second case, on the third day of illness was 10,400 and had dropped to 5,200 on the fifth day. Daily liver extract injections were started on the fifth day and given for 5 days. The general condition of the patient was greatly improved and the average leucocyte count was 7,000. No injections were given on the tenth, eleventh, and twelfth days of the disease. The leucocyte count on the twelfth day was 4,600. Four cubic centimeters of liver extract were given on the thirteenth day, and on the fourteenth day the count rose to 12,200. In three more days the count dropped to 7,000. The temperature was normal by this time, the patient improved rapidly and no complications occurred.

Differential counts coincided with the number of leucocytes. An increased leucocyte count was accompanied by a relative increase in the neutrophiles. The number of white blood cells seemed to be a sufficient index of the patient's condition.

In studying the hospital chart, it was interesting to note that the output of urine was below normal just before the liver therapy was instituted, and greatly increased just after it was given in each case. The first patient averaged an intake of 3,000 c.c. of fluids a day and the second 2,500 c.c. daily.

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EDITORIAL

RENAL FUNCTION TESTS

LABORATORY methods for the estimation of the type and amount of damage that has been done to diseased kidneys are being constantly improved. Classifications of nephritis have been simplified to conform with the data that can be clinically established. Clinical diagnosis can no longer be satisfied with mere qualitative estimation of the presence or absence of albumin and casts in the urine, along with blood pressure determinations or studies and clinical observations. As Addis and his assistants[1] have pointed out, the rate of elimination of protein or serum albumin in the urine as determined by quantitative estimations, along with the quantitative determination of the rate at which formed elements are passed in the urine, is necessary for satisfactory diagnosis and prognosis in renal disease.

Modern tests for the functional capacity of the kidneys will yield information of value not only in the diagnosis and prognosis but also information that is indispensable in the scientific treatment of patients with renal disorders. The

ability of the kidney to concentrate urine specimens, to excrete phenolsulphonephthalein and urea along with the maintenance of normal levels of nonprotein nitrogen waste in the blood plasma, are used in the establishment of renal function. Chapman and Halstead,[2] MacKay and Rytand,[3] Alving and Van Slyke,[4] Freyberg[5] and Stieglitz[6] have made interesting comparative studies of the value of various types of tests. The Van Slyke urea clearance test is generally accepted as the most accurate and most delicate method. It is a standard with which all other tests should be compared, but it requires facilities for microchemical blood and urinalysis.

Alving and Van Slyke[4] have found that the *urinary specific gravity and volume output tests* compare favorably with the urea clearance, provided the tests are made under standard conditions. These simple tests, which have been variously named according to the authors of the test or a modification, as the Volhard, the Addis, the Hedinger and Schlayer, the Mosenthal, are more appropriately designated as the two-hourly renal, the urinary specific gravity, or the concentration and dilutions tests.

If albumin is found in any urine specimen, it should be quantitatively estimated by the Shevky and Stafford[7] modification of the Esbach method, using Tsuchiya's reagent in graduated centrifuge tubes. If there is less than 1 gm. of albumin per liter the effect upon the specific gravity will be negligible. However, every 2.5 gm. of albumin add one point to the third decimal of the specific gravity. Precautions and care are necessary in the taking of the specific gravity.

The two hour and the quarter hour phenolsulphonephthalein excretion tests as introduced by Rowntree and Geraghty[8] have been found by Christian[9] and others to be of limited value, in that they fail to detect or show decreased excretion in the milder cases of nephritis with slight or moderate degrees of impairment. It is often normal after half the functional renal tissue has been destroyed. Furthermore, liver diseases were found to interfere by contributing unreliable, often high, excretion values. Chapman and Halstead[2] recently rediscovered the fact that the curve of elimination of phenolsulphonephthalein was slow to reach the maximum in nephritis and that a delay in the excretion is one of the earliest indications of impaired renal function. The normal 40 per cent was to be excreted within fifteen minutes after intravenous injection. In a large series of cases it was found that in the presence of nephritis there was invariably a decreased fifteen-minute output of the dyestuff and a marked abnormality in the curve of excretion, particularly a delayed peak in the elimination, even when the two-hour output was normal. The technic for performing the test is the same as the usual two-hour test, except that the dye in 1 c.c. amount, containing exactly 6 mg. measured in a tuberculin syringe, is introduced intravenously. The patient is asked to void immediately and drink 600 c.c. of water one-half hour before the injection. A specimen of urine must be obtained at a fifteen-minute interval even if catheterization must be resorted to. Then an hour specimen and again a second hour specimen as the usual test must be obtained. Concentration of the dyestuff in the fifteen-minute, one-hour, and two-hour specimens is determined in a colorimeter, using a standard solution of freshly prepared phenolsulphonephthalein.

Stieglitz and Knight[6] have recently offered as a substitute for the phenolsulphonephthalein test a *sodium ferrocyanide test*, in which the latter substance is introduced intravenously and the output determined quantitatively in the urine. The ferrocyanide is apparently filtered only through the glomeruli. The test, therefore, serves to detect the slightest of pathologic changes in the glomerular membrane. The test seems to have very definite advantages over the phenolsulphonephthalein excretion test, but further studies are necessary to establish the advantages and disadvantages.

Blood and Urine Chemical Tests of Kidney Function.—Facilities for the prosecution of the methods of quantitative blood and urine chemistry are not always available for the general practitioner, but the rise and development of the clinical laboratory in most communities makes it possible for most physicians to avail themselves of these methods of precision. The total blood nonprotein nitrogen levels or those of any one of its constituents as urea, uric acid, creatinine, amino acid, or rest nitrogen have long been considered indices of renal function. The percentages of the total nonprotein nitrogen that a constituent as urea makes up, or the ratio between the two, have been considered by German and French investigators to vary in the presence of renal impairment. Mosenthal and Bruger[10] have recently brought forth substantiating evidence.

The blood urea nitrogen—nonprotein nitrogen ratio, $\frac{\text{BUN}_2}{\text{NPN}} \times 100$ normally remains approximately constant at about 44 or less according to the studies of Mosenthal and Bruger.[10] The ratio rises when renal impairment increases and reaches a level of 80 or more with a maximum impairment as a result of destruction of kidney tissue that has been accomplished. Improvement of the kidney function is evidenced by a drop in ratio, while fixation at a level above 50 and a rapidly rising ratio are to be considered as ill omens. The results of Mosenthal and Bruger[10] compare favorably with the results of the urea clearance tests of Van Slyke. The advantages cited for this test over that of the urea clearance is that only one specimen of blood is necessary and the somewhat troublesome and sometimes difficult collection of urine specimens under basal conditions may be dispensed with.

A review of these modern methods of determining the functional state of the kidneys and the comparative studies and our own experiences leave us with the impression that the simple methods carefully carried out are well worth while. When possible, all of the methods outlined above should be applied and repeated at intervals where there is any question of impairment of the kidney function.

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G. H.

Correspondence

Dear Ed.:

Have you ever noticed that the simplest little discovery may have the most important results? Sir Isaac, for example; if he had seen the apple fall up instead of down we would never have had the Law of Gravity.

I have recently made a simple discovery that may be quite important. I have found that I can nail three medical magazines together as well as three boards.

In our community we have some medical colleagues who would not dream of committing a criminal abortion or of splitting a fee, even by letting the family doctor assist at the operation. But these same honorable gentlemen, when it comes to a loose issue of, say, the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, which contains an article that interests them, display a capacity for the differential diagnosis of mine and thine that is quite lousy.

For many years and particularly during the late (?) depression, when expenditures for bookbinding were out of the question, it was my habit, at quarterly intervals, to bore holes in each set of three journals, string them together with picture wire and solder it in place. We lost fewer journals but it was a monumental task to bind some 45 different journals and the picture wire was rough on the table tops.

Now I nail the journals together. It sounds so simple that it sounds silly. I line three journals up at the edge of a table, turn back the cover, clamp them to the table with a pair of carpenter's clamps for better immobility, and with three wire nails with heads, of a length that will not quite permit of complete penetration, nail them together. Then I turn the journals face down, turn back the back and drive three more nails. Headed wire nails, 1¼, 1, and ¾ inch are best suited. An honest doctor may steal one journal but three together wouldn't go into an overcoat pocket, even if we wore overcoats in Hawaii.

You probably will not publish this letter for I see that you are in the bookbinding business.

At the risk of seeming just too erudite, I add another discovery, simple but with far-reaching consequences; I have discovered that since holding the nail I was driving with a pair of forceps, I haven't hit my thumb once.

Aloha,

E. A. FENNEL.

Honolulu.
February 3, 1936.